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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM:

SUBJECT: Review of data for S-23031 (V-23031) for an EUP for use on corn and soybeans with a temporary tolerance

EPA IDENTIFICATION NUMBERS: P.C. Code: 128723
HED Project Number: 2-1304

FROM: Robert F. Fricke, Ph.D.
Toxicology Branch II, Section IV
Health Effects Division

Robert F. Fricke 7 July 92

TO: Daniel Kenny
Product Manager (74)
Registration Division (H7508C)

THRU: Elizabeth Doyle, Ph.D.
Toxicology Branch II, Head Section IV
Health Effects Division (H7509C)

E. A. Doyle 7/1/92

and

Marcia van Gemert, Ph.D.
Chief, Toxicology Branch II

Marcia van Gemert 7/7/92

Registrant: Valent U.S.A. Corporation
1333 N. California Blvd
Walnut Creek, CA 94596

Chemical: S-23031, V-23031, Pentyl 2-chloro-4-fluoro-5-[(3,4,5,6-tetrahydro)phthalimido] phenoxyacetate)

Action Requested: Review of toxicity data in support of an EUP for S-23031 on corn and soybeans with a temporary tolerance.

Conclusions: The majority of studies submitted by the sponsor met the guideline requirements. Two unacceptable studies dealing with mutagenicity required only supporting documentation to become guideline studies. The skin irritation and sensitization studies, using S-23031, were both classified as unacceptatble (cannot be upgraded) since the test compound was not moistened before testing. Two other acceptable dermal studies, using the formulated product, V-23031 0.83 EC, adequately assessed the skin

1 7/1/92

irritation and sensitization potential. The metabolic studies were the most deficient. The major concern of the reviewer is that all of the major metabolites were not fully identified and quantitated. Although metabolites containing the phenyl moiety were evaluated, those containing the cyclohexenedicarboximide moiety were not. However, the metabolism of other cyclohexenedicarboximide compounds, most notably Captan, indicates that the possible metabolites of S-23031 would be either hydroxylated compounds or amidocarboxycyclohexene, resulting from cleavage of the imide ring. Although specific studies must be upgraded for final registration of the product, the deficiencies noted in the studies were not great enough to prevent the registrant from obtaining a temporary tolerance and EUP for use on corn and soybeans.

Summary of Data

1. Acute Oral Toxicity Study of S-23031 in Mice (81-1), EPA Accession No.: 421698-11

   Male and female mice were exposed orally to test article at doses of 0 and 5000 mg/kg and observed for 14 days for signs of toxicity, mortality and moribundity. All of the animals survived until terminal sacrifice with no abnormal signs. Body weight change was not affected by administration of test article. No remarkable changes related to the test material were observed in gross pathological examination.

   Male and Female: LD_{50} > 5000 mg/kg (limit test)

   Toxicity category IV

   CORE Classification: Guideline

   This study satisfies guideline requirements (81-1) for an acute oral toxicity study in mice.

2. Acute Oral Toxicity Study of S-23031 in Rats (81-1), EPA Accession No.: 421698-12

   Male and female rats were exposed orally to test article at doses of 0 and 5000 mg/kg and observed for 14 days for signs of toxicity mortality and moribundity. All of the animals survived until terminal sacrifice. Neither the males nor the females in the 5000 mg/kg group displayed any abnormal signs. Body weight change was not affected by administration of test article. No remarkable changes related to the test material were observed in gross pathological examination.

   Male and Female: LD_{50} > 5000 mg/kg (limit test)

   Toxicity category IV

   CORE Classification: Guideline

This study satisfies guideline requirements (81-1) for an acute oral toxicity study in the rat.

3. Acute Oral Toxicity to Rats of V-23031 0.83 EC (81-1) EPA Accession No.: 421698-13

Male and female rats were exposed by oral gavage to test compound at doses of 3.2, 4.0, and 5.0 mg/kg and observed for 14 days for signs of toxicity, mortality and moribundity. Clinical signs of toxicity persisted for four days for animals in the 3.2 and 4.0 mg/kg dose groups and for 5 days at the 5.0 mg/kg dose. Mortality was observed in all dose groups. No remarkable changes related to the test material were observed on gross pathological examination at terminal sacrifice.

LD_{50} = 4.1 mg/kg (male), 3.2 mg/kg (female), 3.6 mg/kg (male and female)

Toxicity category III

CORE Classification: Guideline

This study satisfies guideline requirements (81-1) for an acute oral toxicity study in the rat.

4. Acute Dermal Toxicity Study of S-23031 in Rats, EPA Accession No.: 421698-14

S-23031 (2000 mg/kg, suspended in 1% methylcellulose)) or vehicle (1% methylcellulose) was applied to the shaved backs of male and female rats. Animals were observed daily for 14 days for signs of toxicity, mortality and moribundity. All of the animals survived until terminal sacrifice; no abnormal signs were found in either sex at 2000 mg/kg. Body weight change was not affected by administration of test article. No remarkable changes related to the test material were observed in gross pathological examination.

Male and female: LD_{50} > 2000 mg/kg

Toxicity category III

CORE Classification: Guideline

This study satisfies guideline requirements (81-2) for an acute dermal toxicity study in the rat.

5. V-23031 0.83 EC Acute Inhalation Toxicity in Rats, EPA Accession No.: 421698-17

Animals, five rats/sex/group, were randomly distributed to control and treatment groups. Group 1 (control) received air only while Groups 2 through 6 were exposed to a liquid aerosol of V-23031 0.83 EC at concentrations of 5.92, 2.84,

3.63, 5.45, and 4.90 mg/l, respectively, for four hours. Clinical observations were taken at 0.25, 0.5, 1 hour of the equilibration period, hourly during the 4-hour exposure period and twice daily during the 14-day observation period. Rats were weighed daily, starting five days before the start of the study and through the end of the observation period. All animals were necropsied for gross pathological and histopathological examinations.

Male and Female: LC_{50} = 5.51 mg/l

Toxicity category IV

CORE Classification: Guideline

This study satisfies guideline requirements (81-3) for an acute inhalation toxicity study in the rat.

6. Primary Skin Irritation Test with S-23031 in Rabbits, EPA Accession No.: 421698-18

S-23031 did not produce any skin irritation. However, it was applied as a dry powder, which may not have made good contact with the skin. The test compound should have been moistened with water or some other suitable solvent.

CORE Classification: unacceptable (can not be upgraded)

This study does not satisfy guideline requirements (81-5) for a primary skin irritation study in rabbits.

7. Eye Irritation to the Rabbit of V-23031 0.83 EC, EPA Accession No.: 421698-19

The ocular reactions to V-23031 0.83 EC at one hour were limited to conjunctival reddening, chemosis and discharge; conjunctival irritation persisted through observation day 7. After 24 hours, scattered, diffuse opacity of the cornea developed, which persisted through observation day 7. No evidence of iridial irritations was observed. By 14 days no visible irritation was evident in any of the animals.

Toxicity category II

CORE Classification: Guideline

This study satisfies guideline requirements (81-4) for a primary eye irritation study in rabbits.

8. Skin Irritation to Rabbit of V-23031 0.83 EC, EPA Accession No.: 421698-20

Following a single four-hour exposure to V-23031 0.83 EC, skin irritation was present 30 minutes after removal of the test compound; irritation persisted, with progressively

decreasing severity, through day 14. At 4 days edema and erythema were scored as slight and well-defined, respectively. The presence of blanching (slight eschar formation) through day 4 is indicative of severe skin irritation.

Toxicity category II

CORE Classification: Guideline

This study satisfies guideline requirements (81-5) for a primary skin irritation study in rabbits.

9. Skin Sensitization Test with S-23031 in Guinea-Pigs (Buehler's Method), EPA Accession No.: 421698-21

Buehler's method was used to evaluate the skin sensitivity of S-23031 in guinea pigs. Based on the results of the study, the test compound did not exhibit any sensitization potential. However, the test compound was apparently applied as a dry powder, which may not have made good contact with the skin. The test compound should have been moistened sufficiently with water or some other suitable solvent.

CORE Classification: Unacceptable (cannot be upgraded)

This study does not satisfy guideline requirements (81-6) for a dermal sensitization study in guinea pigs.

10. Skin Sensitization Test with S-23031 in Guinea-Pigs (Maximization Method), EPA Accession No.: 421698-22

The maximization method was used to evaluate the skin sensitization potential of S-23031 in guinea pigs. Based on the results of the study, S-23031 was found to be an extreme skin sensitizer.

CORE Classification: Guideline

This study satisfies guideline requirements (81-6) for a dermal sensitization study in guinea pigs.

11. Skin Sensitisation in Guinea-Pig of V-23031 0.83 EC, EPA Accession No.: 421698-23

Buehler's method was used to evaluate the skin sensitivity of S-23031 in guinea pigs. Based on the results of the study, S-23031 did not exhibit any sensitization potential.

CORE Classification: Guideline

This study satisfies guideline requirements (81-6) for a dermal sensitization study in guinea pigs.

12. Skin Sensitisation in Guinea-Pig of V-23031 0.83 EC, EPA Accession No.: 421698-24

The maximization method was used to evaluate the skin sensitization potential of the test compound in guinea pigs. Based on the results of the study, S-23031 was found to be a strong sensitizer with 75% (15/20) of the test animals responding positively.

CORE Classification - Guideline

This study satisfies guideline requirements (81-6) for a dermal sensitization study in guinea pigs.

13. Three-Month Subacute Toxicity Study of S-23031 by Dietary Administration in Rats, EPA Accession Nos.: 421698-26 and 423108-01 (Addendum)

Male and female rats were given test compound daily, for 13 weeks, at dosages of 0, 100, 1000, 10000 or 20000 ppm (equivalent to 0, 6.6, 67.0, 664 or 1359 mg/kg/day for males and 0, 7.4, 73.8, 726 or 1574 mg/kg/day for females, respectively). LOEL is based on increased relative liver weights (male) and increased cholinesterase activity (female).

	NOEL	LOEL
Male	10000 ppm (MDT2)	20000 ppm (LDT)
Female	100 ppm (HDT)	1000 ppm (MDT1)

CORE Classification: Guideline

This study does satisfy guideline requirements (82-1) for a 90-day feeding study in rats.

14. Range-Finding Rabbit Teratology Study with S-23031, EPA Accession Nos.: 421698-29 and 423108-02 (Addendum)

The developmental effects of S-23031 on rabbits was evaluated in a range-finding study. Animals were dosed at 0, 300, 500, 1000 or 1500 mg/kg/day throughout the organogenesis period. The maternal LOEL was based on the increased incidence of mortality. No developmental toxicity was present at the highest dose tested (1500 mg/kg/day).

	NOEL	LOEL
Maternal	500 mg/kg/day	1000 mg/kg/day
Developmental	1500 mg/kg/day	> 1500 mg/kg/day

CORE Classification: Supplementary (This is not a guideline study.)

15. Rabbit Teratology Study with S-23031, EPA Accession No.: 421698-30

The teratological effect of S-23031, at doses of 0, 100, 200, 400 or 800 mg/kg/day, was evaluated in rabbits throughout the organogenesis period. The maternal LOEL was based on the increased incidence of mortality. No developmental toxicity was present at the highest dose tested (800 mg/kg/day).

	NOEL	LOEL
Maternal	400 mg/kg/day	800 mg/kg/day
Developmental	800 mg/kg/day	> 800 mg/kg/day

CORE Classification: Guideline

This study does satisfy guideline requirements (83-3) for Teratology - Developmental Toxicity in the rabbit.

16. Dose-Finding Study for Teratology in Rats with S-23031, EPA Accession No.: 421698-31

The developmental effects of S-23031 on rats was evaluated in a range-finding study. Animals were dosed at 0, 300, 500, 1000 or 1500 mg/kg/day throughout the organogenesis period. No maternal or developmental toxicity was present at the highest dose tested (1500 mg/kg/day).

	NOEL	LOEL
Maternal	1500 mg/kg/day	> 1500 mg/kg/day
Developmental	1500 mg/kg/day	> 1500 mg/kg/day

CORE Classification: Supplementary (This is not a guideline study).

17. Rat Teratology with S-23031, EPA Accession No.: 421698-32

The teratological effects of S-23031 were evaluated in rats dosed at 0, 50, 500 or 1500 mg/kg/day throughout the organogenesis period. No maternal or developmental toxicity was present at the highest dose tested (1500 mg/kg/day).

	NOEL	LOEL
Maternal	1500 mg/kg/day	> 1500 mg/kg/day
Developmental	1500 mg/kg/day	> 1500 mg/kg/day

CORE Classification: Guideline

This study satisfies guideline requirements (83-3) for Teratology - Developmental Toxicity in the rat.

18. A Dietary Dose Range-Finding Reproductive Toxicity Study of S-23031 in Rats, EPA Accession No.: 421698-34

This range-finding study was designed to evaluate the reproductive and systemic toxicity of S-23031 administered to male and female rats for one generation. Dosages of 0, 1000, 5000, 10000 or 20000 ppm (equivalents to: 61, 302,

617 and 1300 mg/kg/day for males and 72, 344, 707 and 1450 mg/kg/day females, prior to breeding; 76, 366, 765 and 1505 mg/kg/day during gestation; 153, 753, 1610 and 3055 mg/kg/day during lactation) did not elicit significant systemic or reproductive toxicity.

	NOEL	LOEL
Systemic	20000 ppm	> 20000 ppm
Reproductive	20000 ppm	> 20000 ppm

CORE Classification: Supplementary (Not a guideline study.)

19. A dietary two-generation reproduction study of S-23031 in rats, EPA Accession No.: 421698-35

The effect of dietary administration of S-23031, at dosages of 0, 200, 10000 or 20000 ppm (respective mg/kg/day equivalents are 16, 878, and 1715 (males) and 18, 829, and 1889 (females) prior to breeding; 14, 746 and 1551 during gestation; 32, 1670 and 3226 during lactation), was studied on the reproductive performance of rats over two generations. The systemic LOEL is based on increased absolute and relative liver and kidney weights at 10000 ppm. Reproductive LOEL is based on increased pup death on Day 0 in the 10000 ppm group.

	NOEL	LOEL
Systemic	200 ppm	10000 ppm
Reproductive	200 ppm	10000 ppm

CORE Classification: Guideline

This study satisfies the guideline (83-4) for reproductive and fertility effects in rats.

20. Salmonella/mammalian activation gene mutation assay, EPA Accession No.: 421698-36

S-23031, at doses of 100, 200, 500, 1000, 2000, and 5000 μg/plate, was not mutagenic in the assay either with of without S-9 activation.

CORE Classification: Unacceptable

(May be upgraded to acceptable if information is provided to indicate that the tester strains were properly maintained and that they were checked for genetic markers.)

This study does not satisfy the guideline (84-2) requirements for a "Gene Mutation".

21. Micronucleus Test of S-23031 in ICR Mice, EPA Accession No.: 421698-37

S-23031, at the highest dose of 5000 mg/kg, was administered

by oral gavage to male and female ICR mice. Compared to the vehicle control, no significant differences in the frequency

of micronucleated cells were noted in the bone marrow cells from the animals treated with S-23031.

CORE Classification: Acceptable

This study satisfies the guideline (84-2) requirements for a "Structural chromosomal aberration test".

22. *In vitro* Chromosomal Aberration Test of S-23031 in Chinese Hamster Ovary Cells (CHO-K1), EPA Accession No.: 421698-38

The results of this study indicate that in the absence of metabolic activation, S-23031 was a weak inducer of chromosomal aberrations; in the presence of metabolic activation, the results were negative.

CORE Classification: Unacceptable

(Study may be upgraded to acceptable if documentation is provided which indicates that the cell cultures were properly maintained and periodically checked for both mycoplasma contamination and karyotype stability.)

This study does not satisfy the guideline (84-2) requirements for a "Structural chromosomal aberration test".

23. *In vitro* Unscheduled DNA Synthesis (UDS) Assay of S-23031 in Rat Hepatocytes, EPA Accession No.: 421698-39

S-23031 did not fulfil either of the evaluation criteria for a positive result. At concentrations up to 300 µg/ml, S-23031 did not elicit unscheduled DNA synthesis in primary cultures of rat hepatocytes.

CORE Classification: Acceptable

This study is acceptable and fulfills the guideline (84-2 (3)) requirements for "Other Genotoxic Effects".

24. Metabolism of S-23031 in Rats Revised, EPA Accession No.: 421698-40

The absorption, distribution, metabolism and excretion of [phenyl-(UL)-^{14}C]-labeled S-23031 was studied on three groups of male and female Sprague-Dawley rats. Two study groups received a single dose by oral gavage of labeled test compound at either 1 mg/kg or 500 mg/kg; the third group was treated daily for 14 days with unlabeled test compound at 1 mg/kg/day by oral gavage, followed on the 15th day with a dose of 1 mg/kg of labeled compound. The test compound is rapidly absorbed and eliminated; after 48 hours, 92.7 to

97.8% of the administered radioactivity was recovered in the urine and feces. The distribution of labeled residues between the urine and feces was comparable in the low and repeat dose groups. However, for animals in the high dose group, fecal elimination of the test compound predominated. The metabolic profiles for were similar for all three study groups. The primary metabolic transformation was deesterification. The deesterified residues were either cleaved of the imide moiety or underwent a series of hydroxylation and/or sulfonation reactions. Compared to the low dose group, the repeat dose group showed slightly higher sulfonation, although the difference was not significant. The tissue accumulation of ^{14}C-labeled residues was very low. Detectable amounts were found in the only kidneys and livers. Accumulation of residues in the kidneys of the females was significantly higher than the males.

CORE Classification: Supplementary.

(Major deficiencies: All metabolites containing the cyclohexenedicarboximide moiety were identified and no information is given in the methods section describing how the two 4-OH-IMCA-SA isomers and 5-OH-IMCA-SA were isolated and identified.)

This study does not satisfy guideline requirements (85-1) for a metabolism study in rats.

25. Combined Chronic Toxicity and Oncogenicity Study of S-23031 by Dietary Administration in Rats (52-Week Interim Report), EPA Accession No.: 421874-06

RESULTS: Male and female rats were given test article daily, for 53 weeks, at dietary concentrations of 0, 100, 1000, 10000 or 20000 ppm (equivalent to approximately 0, 4.2, 41.4, 420.0 and 868.9 mg/kg/day for males and 0, 5.1, 50.8, 518.3, and 1064.4 mg/kg/day for females, respectively). Significant, treatment-related effects, occurring at 10000 and 20000 ppm. The LOEL is based on increased water consumption and urine output in females, decreased alkaline phosphatase activities in both sexes, increased absolute and relative kidney weights of females, and increased relative liver weights in both sexes.

Males and Females: NOEL = 1000 ppm (MDT1) (≈ 50 mg/kg/day)
LOEL = 10000 ppm (MDT2) (≈ 500 mg/kg/day)

CLASSIFICATION: core - Supplementary

This study does not satisfy (Interim Report) guideline requirements (83-5) for a combined chronic toxicity/oncogenicity study in rats.

Reviewed by: Robert F. Fricke, Ph.D. Robert F. Fricke 26 June 92
Section IV, Tox. Branch II (H7509C)
Secondary Reviewer: Elizabeth A. Doyle, Ph.D. E.A. Doyle
Section IV, Tox. Branch II (H7509C) 6/26/92

DATA EVALUATION REPORT

STUDY TYPE: Combined chronic toxicity/oncogenicity studies (interim report) (83-5)

P.C. CODE: 128724

MRID NO.: 421874-06

TEST MATERIAL: S-23031

SYNONYMS: Pentyl 2-chloro-4-fluoro-5-[(3,4,5,6-tetrahydro)phthalimido] phenoxyacetate

STUDY NUMBER: 1716

SPONSOR: Valent U.S.A. Corporation
1333 N. California Blvd
Walnut Creek, CA 94596

TESTING FACILITY: Sumitomo Chemical Co., Ltd
Environmental Health Science Laboratory
1-98, 3-Chome
Kasugade-naka, Konohana-ku
Osaka, Japan

TITLE OF REPORT: Combined Chronic Toxicity and Oncogenicity Study of S-23031 by Dietary Administration in Rats (52-Week Interim Report)

AUTHOR: H. Adachi

REPORT ISSUED: 27 December 1990

CONCLUSIONS: Male and female rats were given test article daily, for 53 weeks, at dietary concentrations of 0, 100, 1000, 10000 or 20000 ppm (equivalent to approximately 0, 4.2, 41.4, 420.0 and 868.9 mg/kg/day for males and 0, 5.1, 50.8, 518.3, and 1064.4 mg/kg/day for females, respectively). Significant, treatment-related effects, occurred at 10000 and 20000 ppm and consisted of increased water consumption and urine output in females, decreased alkaline phosphatase activities in both sexes, increased absolute and relative kidney weights of females, and increased relative liver weights in both sexes.

Males and Females: NOEL = 1000 ppm (MDT1) (≈ 50 mg/kg/day)
LOEL = 10000 ppm (MDT2) (≈ 500 mg/kg/day)

CLASSIFICATION: core - Supplementary

This study does not satisfy (Interim Report) guideline requirements (83-5) for a combined chronic toxicity/oncogenicity study in rats.

A. MATERIALS:

1. Test compound: S-23031, technical Description: brown powder Batch #: PYG-88092-M Purity: 94.7% Contaminants: list in CBI appendix.

2. Test animals: Species: Rat Strain: Crj:CD(SD) Age: 5 weeks Weight (g): 164 - 213 (males), 135 - 191 (females) Source: Charles River Japan, Inc.

B. STUDY DESIGN:

1. Animal assignment: Animals were assigned randomly to main and satellite study groups as shown in Table 1. The animals in the satellite group were sacrificed after 52 weeks.

Table 1: Animal Assignment to Study Groups

Test Group	Dose in Diet (ppm)	Main Group (104 Weeks) Male	Main Group (104 Weeks) Female	Satellite Group (52 Weeks) Male	Satellite Group (52 Weeks) Female
Control (CON)	0	50	50	14	14
Low (LDT)	100	50	50	14	14
Mid1 (MDT1)	1000	50	50	14	14
Mid2 (MDT2)	10000	50	50	14*	14
High (HDT)	20000	50	50	12	14

* Two animals were removed from the study because of technician error.

2. Diet preparation: For preparation of the test diets, a premix (one for each dose group) was made using an appropriate amount of test article and thoroughly mixing it with the basal diet. Additional basal diet was added to the different premixes to form the correct dietary concentration of test article. Analysis of samples, taken from the top, middle and bottom of the prepared diets, showed that the test article was homogeneously distributed (coefficient of variation 0.29 to 0.60%) and within 96.9 and 100% of nominal concentration. Test diets were stable for six weeks in the refrigerator and four weeks at room temperature.

3. Animals received diet (CRF-1, Oriental Yeast Co., Ltd., Tokyo, Japan) and water *ad libitum*. For determination of water consumption, water was administered using water bottles.

4. Statistics: A one-way analysis of variance (ANOVA) was performed on body weight, food consumption, water consumption, hematology, blood chemistry and organ weight. If a significant ANOVA result was found, pair-wise comparisons were carried out using the Least Significant

Difference test. Scheffe's mean rank test was used to test for significant differences in the urinalysis data; significant data were further analyzed using the Kruskal-Wallis analysis of ranks. No statistical procedure was presented for the analysis of incidence data for clinical observations, gross pathological or histopathological observations.

5. Quality assurance was documented by signed and dated GLP and quality assurance statements.

6. The sponsor applied the criteria of 40 CFR 158.34 for flagging studies for potential adverse effects to the results of this study. This study neither meets nor exceeds any of the applicable criteria.

C. METHODS AND RESULTS:

1. Observations: Animals in the main study group were inspected twice (weekdays) or once (weekends, holidays) daily for signs of toxicity, moribundity and mortality. Animals in the satellite study group were inspected once daily.

a. Toxicity: The only clinical observations attributable to the administration of the test article were occasional urine incontinence and staining of the tails of animals in the 20000 ppm groups. Significant findings were also observed in the 10000 ppm group, however, the occurrence was infrequent. For males in the main study, the incidence of staining was significantly higher from Week 7 through 30. From Weeks 31 through 44 and Week 49 through 53, staining was present, but not statistically different from control values. For males in the satellite group, occasional, but insignificant, staining was observed. Similar to the males, the females in the main study showed significant staining in the 20000 ppm group from Week 7 through 53; occasional significant findings were observed in the 10000 ppm group at Weeks 7 to 9, 21, 22, 34, 35, 38 to 42, 47 to 49, 52, and 53. Significant staining was observed in females in the satellite study from Weeks 26 through 35; staining was present from Weeks 37 through 52, but the incidence rate was not significantly different from control values. The only other clinical sign of significance was urinary incontinence, which was present only in females for weeks 23, 30, 31, 34, 35, 40, and 41. Occasional, but insignificant incidence of urinary incontinence was observed primarily in the 20000 ppm group, and to a lesser extent in the 10000 ppm group.

b. Mortality (survival): Cumulative mortality through Week 53 is summarized in Table 2. Although mortality was observed in all of the study groups, there was no

dose-related pattern to suggest a treatment-related effect. Also, there was no consistent, underlying cause of death, suggesting that none of the deaths could be attributed to treatment.

Table 2: Animal Mortality through Week 53 (Data summarized from Table 1 of the study)

Study Group	Main Group		Satellite Group	
	Male	Female	Male	Female
CON	2	0	1	1
LDT	1	1	0	0
MDT1	2	0	1	0
MDT2	3	1	3	0
HDT	1	0	0	0

2. Body weight: Animals in the main study were weighed at the start of the study, once weekly for 14 weeks and every four weeks thereafter. Animals in the satellite study were weighed every 4 weeks. All animals were weighed at terminal sacrifice.

Results: For male animals in both the main and satellite study groups, no significant differences in body weight were observed throughout the study. The only significant effects were increases in mean body weight for females in the 100 ppm group for days 8 through 365 in the main study and days 176 through 365 for the satellite study. Since similar significant findings were not observed at higher doses of test compound, the effects were not attributed to treatment.

3. Food and water consumption and compound intake: Food consumption, measured over 6 or 7 consecutive days, was determined weekly through week 14 and once every four weeks for the remainder of the study. For animals in the satellite study, food consumption was measured every four weeks. Ten animals in each dose group were selected for measurement of water consumption. The absolute and relative water consumption was measured for 48 consecutive hours during Weeks 25 to 26 and 53.

a. Food consumption results: Significant increases in absolute (g/animal/day) and relative (g/kg body weight/day) food consumption were noted throughout the study in both males and females. The observed differences were, however, slight and not of biological significance. Further, there was a lack of dose-dependency and the occurrence was sporadic in nature.

c. Compound intake: The mean compound intake is summarized in Table 3, below.

Table 3 Compound Intake (mg/kg body weight/day) for Weeks 1-53 (Data summarized from Table 6 of the study)

Study Group	Main Group		Satellite Group	
	Male	Female	Male	Female
LDT	4.2	5.1	4.1	5.1
MDT1	41.4	50.8	40.3	49.8
MDT2	420.0	518.3	415.6	514.1
HDT	868.9	1064.4	851.1	1051.5

c. Water consumption: While the males did not show any significant changes in water consumption, the females showed significant, treatment-related increases at both 10000 and 20000 ppm (Table 4).

Table 4: Absolute (ml/animal/day) and Relative (ml/kg body weight/day) Water Consumption by Female Rats (Data summarized from Tables 7 and 8 of the study)

	Day	CON	LDT	MDT1	MDT2	HDT
Absolute	177	36	33	34	74**	52
	366	44	38	40	63**	58*
Relative	177	110.2	105.8	109.9	231.1**	169.0*
	366	112.5	106.0	107.8	163.3**	156.5*

* $p < 0.05$, ** $p < 0.01$

4. Ophthalmological examinations: Examinations were performed on control and high dose animals in the main study group before the study was initiated and during Week 54 of treatment. No treatment-related eye lesions were observed.

5. Urinalysis: Urinalysis was performed during Weeks 25 to 27 on 10 animals/sex/dose selected from the satellite study group. The checked (X) parameters were examined.

X Volume	X Glucose
X Specific gravity	X Ketone Bodies
X Protein	Bile Pigments
X Appearance	X Urobilirubin
X Sediment	X Total Bilirubin
X pH	X Occult Blood

Results: Compared to the control value of 2.0 ml, significant ($p \leq 0.05$) increases in urine volume were observed in females at 10000 and 20000 ppm (4.2 and 4.1 ml, respectively).

6. Hematology and Clinical Chemistry: Ten animals/dose/sex, selected from the satellite study group, were subjected to hematological and biochemical analyses during Weeks 27 and 53. The checked (X) parameters were examined.

a. Hematology

X Hematocrit (HCT)
X Hemoglobin (HGB)
X Leukocyte count (WBC)
X Erythrocyte count (RBC)
X Platelet count
Bone marrow
Prothrombin time
X Leukocyte differential count
X Mean corpuscular HGB (MCH)
X Mean corpuscular HGB conc. (MCHC)
X Mean corpuscular volume (MCV)

Results Hematology results of the 27- and 53-week interim examinations revealed no biologically meaningful differences between the control and treatment groups.

b. Clinical Chemistry

Electrolytes
X Calcium
X Chloride
Magnesium
X Phosphorous
X Potassium
X Sodium

Enzymes
X Alkaline phosphatase
X Plasma Cholinesterase
X Creatinine phosphokinase
X Lactic acid dehydrogenase
X Leucine Aminopeptidase
X γ-Glutamyl transpeptidase
X Serum alanine aminotransferase (SGPT/ALT)
X Serum aspartate aminotransferase (SGOT/AST)

Other
X Albumin
X Blood creatinine
X Blood urea nitrogen
X Total cholesterol
X Globulins
X Glucose
X Total Bilirubin
X Triglycerides
X Total Protein
X Phospholipid
X Direct Bilirubin
X Serum Protein Fractionation

Results: Significant clinical chemistry results, determined at 27 and 53 weeks, are summarized in Table 5. Treatment-related decreases in alkaline phosphatase occurred at 20000 ppm in males and 10000 ppm in females at 27 weeks and in both sexes at 53 weeks. Possible treatment-related increase in the γ-globulin and decrease in α_1-globulin were noted in males in the 20000 ppm group at 27 weeks. Other significant findings were observed in other clinical chemistry parameters, however, they were either not dose-related or the differences were too slight to be of biological significance.

Table 5: 27- and 53-Week Clinical Chemistry Results (Data summarized from Table 11 of the study)

Parameter	Sex	Week of Study	CON	LDT	MDT1	MDT2	HDT
Alkaline Phosphatase (U/l)	Male	27	102	101	102	89	81**
	"	53	102	93	93	77**	78**
Alkaline Phosphatase (U/l)	Female	27	44	41	42	30**	29**
	"	53	48	31**	38	27**	25**
γ-Globulin (%)	Male	27	16.7	17.5	17.5	17.9	19.8**
α_1-Globulin (%)	Male	27	8.6	8.4	8.6	8.2	7.0**

** $p \leq 0.01$

7. Sacrifice and Pathology: Ten animals/dose/sex, selected from the satellite group, were subjected to detailed pathological examination during Week 53. Additionally all moribund sacrifices and animals which died during the study were subjected to histopathological examination. The checked (X) tissues were collected for histological examination; the checked (XX) organs were also weighed.

Digestive system
X Tongue
X Salivary glands
X Esophagus
X Stomach
X Duodenum
X Jejunum
X Ileum
X Cecum
X Colon
X Rectum
XX Liver
Gall bladder
X Pancreas

Respiratory
X Trachea
X Lungs
Nasal Passages

Cardiovas./Hematol
X Aorta
XX Heart
X Bone marrow
X Lymph nodes
XX Spleen
X Thymus

Urogenital
XX Kidneys
X Urinary bladder
XX Testes
X Epididymides
XX Prostate
X Seminal vesicle
XX Ovaries
X Uterus
X Vagina
Cervix

Neurologic
XX Brain
X Periph. nerve
X Spinal cord
XX Pituitary
X Eyes

Glandular
XX Adrenals
Lacrimal gland
X Mammary gland
XX Parathyroids
XX Thyroids

Other
X Bone
X Skeletal muscle
X Skin
X Gross lesions
X Harderian glands

a. Organ Weights: Significant findings for the absolute and relative organ weights at the interim sacrifice are shown in Table 6, below. The relative liver weights were significantly increased in both males and females at 10000 ppm. Relative kidney weights were increased at 20000 ppm for males and 10000

ppm for females. Since the mean body weights of the males were lower in the treated groups, the lower absolute spleen weights were not judged to be treatment-related; no statistically significant differences were found in the relative spleen weights.

Table 6: Absolute (g) and Relative (g/kg body wt) Organ Weights (Data summarized from Tables 13 and 14 of the study)

Observation	Sex	CON	LDT	MDT1	MDT2	HDT
Absolute Organ Weights						
Spleen	Male	1.02	0.85*	0.85*	0.81**	0.83**
Kidney	Female	2.30	2.47	2.28	2.60*	2.76**
Relative Liver Weights						
Liver	Male	2.25	2.37	2.19	2.45*	2.66**
	Female	2.21	2.37	2.31	2.50**	2.63**
Kidney	Male	0.60	0.60	0.58	0.64	0.67*
	Female	0.62	0.73**	0.64	0.71*	0.78**

* $p < 0.05$, ** $p < 0.01$

b. Gross Pathology: Significant gross pathological changes, observed only in the males, consisted of enlarged livers in the 20000 ppm group; in the females no treatment-related effects were observed.

c. Microscopic Pathology

1) Non-neoplastic: Although histopathological lesions were observed in both the control and treatment groups, none was considered to be treatment-related.

2) Neoplastic: Not noted

D. DISCUSSION: This study evaluated the interim, 53-week results for a 104 week combined chronic toxicity and oncogenicity study in rats. Male and female rats were given test article daily, at dietary concentrations of 0, 100, 1000, 10000 or 20000 ppm (equivalent to approximately 0, 4.2, 41.4, 420.0 and 868.9 mg/kg/day for males and 0, 5.1, 50.8, 518.3, and 1064.4 mg/kg/day for females, respectively).

Significant, treatment-related effects occurring during the study were limited. Clinical findings consisted of urine incontinence

and staining of the tails of animals in the 20000 ppm group and infrequently in the 10000 ppm group.

Significant increases in both absolute and relative food consumption were noted throughout the study in both males and females. The observed differences were, however, slight and not considered to be of biological significance. While the males did not show any significant changes in water consumption, the females showed significant, treatment-related increases at both 10000 and 20000 ppm. Increased water consumption in the females correlated with a significant increase in urine output.

Significant findings observed at the interim sacrifice were limited to decreased alkaline phosphatase activities in both sexes, increased absolute and relative kidney weights of females, and increased relative liver weights in both sexes.

Males and Females: NOEL = 1000 ppm (MDT1) (≈ 50 mg/kg/day)
LOEL = 10000 ppm (MDT2) (≈ 500 mg/kg/day)

Classification: core - Supplementary

This study does not satisfy (Interim Report) guideline requirements (83-5) for a combined chronic toxicity/oncogenicity study in rats.

Reviewed by: Robert F. Fricke, Ph.D. Robert F. Fricke 26 Mar 92
Section IV, Tox. Branch II (H7509C)
Secondary Reviewer: Elizabeth A. Doyle, Ph.D. E. A. Doyle 3/26/92
Section IV, Tox. Branch II (H7509C)

DATA EVALUATION REPORT

009587

STUDY TYPE: Acute Oral Toxicity - Mouse (81-1)

P.C. CODE: 128723

MRID NO.: 421698-11

TEST MATERIAL: S-23031

SYNONYMS: Pentyl 2-chloro-4-fluoro-5-[(3,4,5,6-tetrahydro)phthalimido]phenoxy acetate

STUDY NUMBER: 2005

SPONSOR: Valent U.S.A. Corporation
1333 N. California Blvd
Walnut Creek, CA 94596

TESTING FACILITY: Sumitomo Chemical Co., Ltd
Biochemistry and Toxicology Laboratory
1-98, 3-Chome
Kasugade-naka, Konohana-ku
Osaka, Japan

TITLE OF REPORT: Acute Oral Toxicity Study of S-23031 in Mice

AUTHOR: T. Hiromori

REPORT ISSUED: 2 February 1990

CONCLUSIONS: Male and female mice were exposed orally to test article at doses of 0 and 5000 mg/kg and observed for 14 days for signs of toxicity, mortality and moribundity. All of the animals survived until terminal sacrifice with no abnormal signs. Body weight change was not affected by administration of test article. No remarkable changes related to the test material were observed in gross pathological examination.

Male: LD_{50} > 5000 mg/kg (limit test)

Female: LD_{50} > 5000 mg/kg (limit test)

Toxicity category IV

Classification: core - Guideline

This study does satisfy guideline requirements (81-1) for an acute oral toxicity study in the mouse.

MATERIALS AND METHODS:

Test compound: S-23031 Description: White to light brown solid Batch #: PYG-88092-M Purity: 94.7% Contaminants: list in CBI appendix.

Test animals: Species: Mouse Strain: ICR Age: 6 weeks Weight (g): 26.5 - 29.9 (males), 17.2 - 21.6 (females) Source: Charles River Japan, Inc.

Study design: Animals were randomly assigned to control and treatment groups. Each group consisted of five males and five females. Test article was suspended in 1% methylcellulose at a concentration of 250 mg/ml and administered in a dose volume of 20 ml/kg. Animals were given either 0 (vehicle) or 5000 mg/kg test article by gavage. Animals were observed for signs of toxicity, moribundity and mortality immediately after dosing (1/6, 1/2, 1, 2, 4 hours) and daily, thereafter, for 14 days. Animals were weighed on days 0, 7, and 14 of the observation period. At the end of the observation period animals were necropsied for gross pathological examination.

Statistics: Means and standard deviations were calculated for animal body weights and body weight gains. Significant differences were evaluated using Student's t-test. Incidence of gross pathological lesions were evaluated using the Fisher's exact test.

RESULTS AND DISCUSSION

Animals were inspected daily for signs of toxicity, moribundity and mortality. There were no treatment-related changes in either clinical signs, body weights, body weight gains, or gross pathological findings.

CONCLUSIONS: Male and female rats were exposed orally to test article at doses of 0 and 5000 mg/kg and observed daily for 14 days for signs of toxicity, mortality and moribundity. All of the animals survived until terminal sacrifice. No abnormal signs were found in any sex at 5000 mg/kg. Body weight change was not affected by administration of test article. No remarkable changes related to the test material were observed in gross pathological examination.

Male: LD_{50} > 5000 mg/kg (limit test)

Female: LD_{50} > 5000 mg/kg (limit test)

Toxicity category IV

Classification: core - Guideline

This study does satisfy guideline requirements (81-1) for an acute oral toxicity study in the mouse.

009587

Reviewed by: Robert F. Fricke, Ph.D. Robert F. Fricke 26 March 92.
Section IV, Tox. Branch II (H7509C)
Secondary Reviewer: Elizabeth A. Doyle, Ph.D. E-A-Doyle 3/26/92
Section IV, Tox. Branch II (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: Acute Oral Toxicity - Rat (81-1)

P.C. CODE: 128724

MRID NO.: 421698-12

TEST MATERIAL: S-23031

SYNONYMS: Pentyl 2-chloro-4-fluoro-5-[(3,4,5,6-tetrahydro)phthalimido]phenoxy acetate

STUDY NUMBER: 2000 (Ref. No. SAT-00-0009)

SPONSOR: Valent U.S.A. Corporation
1333 N. California Blvd
Walnut Creek, CA 94596

TESTING FACILITY: Sumitomo Chemical Co., Ltd
Biochemistry and Toxicology Laboratory
1-98, 3-Chome
Kasugade-naka, Konohana-ku
Osaka, Japan

TITLE OF REPORT: Acute Oral Toxicity Study of S-23031 in Rats

AUTHOR: T. Hiromori

REPORT ISSUED: 28 December 1989

CONCLUSIONS: Male and female rats were exposed orally to test article at doses of 0 and 5000 mg/kg and observed for 14 days for signs of toxicity mortality and moribundity. All of the animals survived until terminal sacrifice. Neither the males nor the females in the 5000 mg/kg group displayed any abnormal signs. Body weight change was not affected by administration of test article. No remarkable changes related to the test material were observed in gross pathological examination.

Male and Female: LD_{50} > 5000 mg/kg (limit test)

Toxicity category IV

Classification: core - Guideline

This study does satisfy guideline requirements (81-1) for an acute oral toxicity study in the rat.

MATERIALS AND METHODS:

Test compound: S-23031 Description: White to light brown solid Batch #: PYG-88092-M Purity: 94.7% Contaminants: list in CBI appendix.

Test animals: Species: Rat Strain: Sprague Dawley Age: Weight (g): 196 - 218 (males), 152 - 165 (females) Source: Charles River Japan, Inc.

Study design: Animals were randomly assigned to control and treatment groups. Each group consisted of five males and five females. Test article was suspended in 1% methylcellulose at a concentration of 250 mg/ml and administered in a dose volume of 20 ml/kg. Animals were given either 0 (vehicle) or 5000 mg/kg test article by gavage. Animals were observed for signs of toxicity, moribundity and mortality immediately after dosing (1/6, 1/2, 1, 2, 4 hours) and daily, thereafter, for 14 days. Animals were weighed on days 0, 7, and 14 of the observation period. At the end of the observation period animals were necropsied for gross pathological examination.

Statistics: Means and standard deviations were calculated for animal body weights and body weight gains. Significant differences were evaluated using Student's t-test. Incidence of gross pathological lesions were evaluated using the Fisher's exact test.

RESULTS AND DISCUSSION

Animals were inspected daily for signs of toxicity, moribundity and mortality. There were no treatment-related changes on either clinical signs, body weights, body weight gains, or gross pathological findings.

CONCLUSIONS: Male and female rats were exposed orally to test article at doses of 0 and 5000 mg/kg and observed for 14 days for signs of toxicity mortality and moribundity. All of the animals survived until terminal sacrifice. Neither the males nor the females in the 5000 mg/kg group displayed any abnormal signs. Body weight change was not affected by administration of test article. No remarkable changes related to the test material were observed in gross pathological examination.

Male and Female: LD_{50} > 5000 mg/kg (limit test)

Toxicity category IV

Classification: core - Guideline

This study does satisfy guideline requirements (81-1) for an acute oral toxicity study in the rat.

Reviewed by: Robert F. Fricke, Ph.D. Robert F. Fricke 22 May 92
Section IV, Tox. Branch II (H7509C)
Secondary Reviewer: Elizabeth A. Doyle, Ph.D. E A. Doyle
Section IV, Tox. Branch II (H7509C)
009587 5/22/92

DATA EVALUATION REPORT

STUDY TYPE: Acute Oral Toxicity - Rat (81-1)

P.C. CODE: 128724

MRID NO.: 421698-13

TEST MATERIAL: V-23031 0.83 EC

SYNONYMS: Pentyl 2-chloro-4-fluoro-5-[(3,4,5,6-tetrahydro)phthalimido]phenoxy acetate

STUDY NUMBER: 901090D/VLT 1/AC

SPONSOR: Valent U.S.A. Corporation
1333 N. California Blvd
Walnut Creek, CA 94596

TESTING FACILITY: Huntingdon Research Centre Ltd.
P.O. Box
Huntingdon, Cambridgeshire
PE18 6ES, England

TITLE OF REPORT: Acute Oral Toxicity to Rats of V-23031 0.83 EC

AUTHOR: P. Baldrick and G. Healing

REPORT ISSUED: 13 November 1991

CONCLUSIONS: Male and female rats were exposed by oral gavage to the test compound at doses of 3.2, 4.0, and 5.0 mg/kg and observed for 14 days for signs of toxicity, mortality and moribundity. Clinical signs of toxicity persisted for four days for animals in the 3.2 and 4.0 mg/kg dose groups and for 5 days at the 5.0 mg/kg dose. Mortality was observed in all dose groups. No remarkable changes related to the test material were observed on gross pathological examination at terminal sacrifice.

Male: LD_{50} = 4.1 mg/kg

Female: LD_{50} = 3.2 mg/kg

Male and Female: LD_{50} = 3.6 mg/kg

Toxicity category III

Classification: core - Guideline

This study satisfies guideline requirements (81-1) for a acute oral toxicity study in the rat.

MATERIALS AND METHODS:

Test compound: V-23031 0.83 EC Description: clear amber liquid
Batch #: V 803L01 Purity: 10% Contaminants: not given.

Test animals: Species: Rat Strain: Sprague-Dawley Crl. CD (SD) BR VAF plus Age: 4 - 6 weeks Weight (g): 97 - 129 Source: Charles River U.K., Ltd.

Study design: Animals (five male and five females) were randomly assigned to treatment groups. Groups were dosed by oral gavage at doses of 3.2, 4.0, and 5.0 mg/kg in dose volumes of 3.33, 4.17, and 5.21 ml/kg, respectively. During Day 1, animals were observed soon after dosing and frequently for six hours; for the next 14 days, observations were made twice daily. Animals were weighed on days 1, 8 and 15 of the observation period. At the end of the observation period, all surviving animals were necropsied for gross pathological examination.

Statistics: Probit analysis was used to determine the acute LD_{50} values. The probit curve for males and females was determined and if found to be parallel by chi-square analysis, the best-fit common slope was used to determine the individual male and female LD_{50} values. The mean animal body weight was also determined.

RESULTS AND DISCUSSION

Clinical Signs: Animals were inspected daily for signs of toxicity, moribundity and mortality. Significant clinical signs present immediately after dosing (10 min) included pilo-erection and increased salivation. During Day 1 all rats showed pilo-erection, which was accompanied by hunched posture, abnormal gait, lethargy, decreased respiration rate, ptosis and pallor of the extremities. Clinical signs persisted through Day 4 for the animals in the 3.2 and 4.0 mg/kg dose groups and Day 5 for those in the 5.0 mg/kg group.

Body Weights: Without the inclusion of a control group in the study, the evaluation of treatment-related effects on mean body weight and body weight gain is not possible. Further, the number of surviving animals was not large enough to allow a statistical comparison. However, according to the study, "Slightly low bodyweight gains were recorded for a majority of males and two females dosed at 3.2 mg/kg on Day 8; remaining rats achieved anticipated gains during this period. All rats achieved anticipated bodyweight gains during the second week of the study."

Gross Pathological Examination: Gross pathological examination did not reveal any abnormalities.

Determination of LD_{50} Values: Deaths occurred in both males and females on Days 2 and 3 of the study. The LD_{50} values are summarized in Table 1.

Table 1: Acute Oral LD_{50} (mg/kg) and 95% Confidence Intervals for Male and Female Rats

	LD_{50}	95 % C.I.
Male and Female	3.6	1.9 to 4.4
Male only	4.1	3.0 to 6.1
Female only	3.2	1.2 to 4.0

CONCLUSIONS: Male and female rats were exposed by oral gavage to test article at doses of 3.2, 4.0, and 5.0 mg/kg and observed for 14 days for signs of toxicity, mortality and moribundity. Clinical signs of toxicity persisted for four days for animals in the 3.2 and 4.0 mg/kg dose groups and for 5 days at the 5.0 mg/kg dose. Mortality was observed in all dose groups. No remarkable changes related to the test material were observed on gross pathological examination at terminal sacrifice.

Male: LD_{50} = 4.1 mg/kg

Female: LD_{50} = 3.2 mg/kg

Male and Female: LD_{50} = 3.6 mg/kg

Toxicity category III

Classification: core - Guideline

This study satisfies guideline requirements (81-1) for a acute oral toxicity study in the rat.

Reviewed by: Robert F. Fricke, Ph.D. Robert F. Fricke 19 May 92
Section IV, Tox. Branch II (H7509C)
Secondary Reviewer: Elizabeth A. Doyle, Ph.D. E.A. Doyle
Section IV, Tox. Branch II (H7509C)

DATA EVALUATION REPORT

5/20/92
009587

STUDY TYPE: Acute Dermal Toxicity - Rats (81-2)

P.C. CODE: 128724

MRID NO.: 421698-14

TEST MATERIAL: S-23031

SYNONYMS: Pentyl 2-chloro-4-fluoro-5-[(3,4,5,6-tetrahydro)phthalimido]phenoxy acetate

STUDY NUMBER: 2002

SPONSOR: Valent U.S.A. Corporation
1333 N. California Blvd
Walnut Creek, CA 94596

TESTING FACILITY: Sumitomo Chemical Co., Ltd
Biochemistry and Toxicology Laboratory
1-98, 3-Chome
Kasugade-naka, Konohana-ku
Osaka, Japan

TITLE OF REPORT: Acute Dermal Toxicity Study of S-23031 in Rats

AUTHOR: T. Hiromori

REPORT ISSUED: 28 December 1989

CONCLUSIONS: Test article (2000 mg/kg, suspended in 1% methylcellulose) or vehicle (1% methylcellulose) was applied to the shaved backs of male and female rats. Animals were observed daily for 14 days for signs of toxicity, mortality and moribundity. All of the animals survived until terminal sacrifice; no abnormal signs were found in either sex at 2000 mg/kg. Body weight change was not affected by administration of test article. No remarkable changes related to the test material were observed in gross pathological examination.

Male: LD_{50} > 2000 mg/kg Female: LD_{50} > 2000 mg/kg

Toxicity category III

Classification: core - Guideline

This study does satisfy guideline requirements (31-2) for an acute dermal toxicity study in the rat.

MATERIALS AND METHODS: 009587

Test compound: S-23031 Description: White to light brown solid Batch #: PYG-88092-M Purity: 94.7% Contaminants: list in CBI appendix.

Test animals: Species: Rat Strain: Sprague Dawley Age: 7 weeks Weight (g): 225 - 257 (males), 157 - 192 (females) Source: Charles River Japan, Inc.

Study design: Animals were randomly assigned to control and treatment groups; each group consisted of five males and five females. Either 1 % methylcellulose (vehicle control) or a 250 mg/ml suspension of test article in 1 % methylcellulose was spread over approximately a 30 cm^2 of dorsal area (sheared one day before dosing) at a rate of 8.0 ml/kg body weight. The treated animals received a dose of 2000 mg/kg. Animals were observed immediately after dosing (1/6, 1/2, 1, 2 and 4 hours) and daily, thereafter, for 14 days, for signs of toxicity, moribundity and mortality. Animals were also weighed on days 0, 7 and 14 of the observation period. At the end of the observation period animals were necropsied for gross pathological examination.

Statistics: Means and standard deviations were calculated for animal body weights and body weight gains. Significant differences were evaluated using Student's t-test. Incidence of gross pathological lesions were evaluated using the Fisher's exact test.

RESULTS AND DISCUSSION

Animals were inspected daily for signs of toxicity, moribundity and mortality. There were no treatment-related changes in either clinical signs, body weights, body weight gains or gross pathological findings.

Conclusions: Test article (2000 mg/kg, suspended in 1% methylcellulose) or vehicle (1% methylcellulose) was applied to the shaved backs of male and female rats. Animals were observed daily for 14 days for signs of toxicity, mortality and moribundity. All of the animals survived until terminal sacrifice; no abnormal signs were found in either sex at 2000 mg/kg. Body weight change was not affected by administration of test article. No remarkable changes related to the test material were observed in gross pathological examination.

LD_{50} > 2000 mg/kg (male and female)

Toxicity category III

Classification: core - Guideline

This study does satisfy guideline requirements (81-2) for an acute dermal toxicity study in the rat.

Reviewed by: Robert F. Fricke, Ph.D. Robert F. Fricke 22 May 92
Section IV, Tox. Branch II (H7509C)
Secondary Reviewer: Elizabeth A. Doyle, Ph.D. E. A. Doyle 5/22/92
Section IV, Tox. Branch II (H7509C)

DATA EVALUATION REPORT 009587

STUDY TYPE: Acute Dermal Toxicity - Rats (81-2)

P.C. CODE: 128724

MRID NO.: 421698-15

TEST MATERIAL: V-23031 0.83 EC

SYNONYMS: Pentyl 2-chloro-4-fluoro-5-[(3,4,5,6-tetrahydro)phthalimido]phenoxy acetate

STUDY NUMBER: 901091D/VLT 2/AC

SPONSOR: Valent U.S.A. Corporation
1333 N. California Blvd
Walnut Creek, CA 94596

TESTING FACILITY: Huntingdon Research Centre Ltd.
P.O. Box
Huntingdon, Chambridgeshire
PE18 6ES, England

TITLE OF REPORT: Acute Dermal Toxicity to Rats of V-23031 0.83 EC

AUTHOR: P. Baldrick and G. Healing

REPORT ISSUED: 13 November 1991

CONCLUSIONS: Test article (2000 mg/kg) was applied to the shaved backs of male and female rats. Animals were observed for 14 days for signs of toxicity, mortality and moribundity. All of the animals survived until terminal sacrifice; no abnormal clinical signs were found in either sex. No remarkable changes related to the test material were observed on gross pathological examination at terminal sacrifice.

Male: LD_{50} > 2000 mg/kg Female: LD_{50} > 2000 mg/kg

Toxicity category III

Classification: core - Guideline

This study does satisfy guideline requirements (81-2) for an acute dermal toxicity study in the rat.

MATERIALS AND METHODS:

A. Test compound: V-23031 0.83 EC Description: clear amber liquid Batch #: V 803L01 Purity: 10% Contaminants: not given.

B. Test animals: Species: Rat Strain: Sprague-Dawley Crl. CD (SD) BR Age: 7 - 10 weeks Weight (g): 227 - 275 Source: Charles River U.K., Ltd.

Study design: Animals (five males and five females) were assigned to a single treatment group. The animals received a single dermal dose of test compound (2000 mg/kg), spread over a 25 x 25 mm area of the dorso-lumbar region. The application site was covered with gauze and then covered with an occlusive dressing. After 24-hour exposure the dressing was removed, the skin cleaned with water and blotted dry. Observations were made frequently for seven hours after removal of the dressing and twice daily for 14 days, thereafter. Animals were weighed on days 1, 8 and 15 of the observation period. At the end of the observation period animals were necropsied for gross pathological examination.

Statistics: Mean body weights were determined.

RESULTS AND DISCUSSION

Results: There were no treatment-related changes in either clinical signs or gross pathological findings. All animals survived until the scheduled sacrifice.

Conclusions: Test article (2000 mg/kg) was applied to the shaved backs of male and female rats. Animals were observed daily for 14 days for signs of toxicity, mortality and moribundity. All of the animals survived until terminal sacrifice; no abnormal clinical signs were found in either sex. No remarkable changes related to the test material were observed on gross pathological examination.

LD_{50} > 2000 mg/kg (male and female)

Toxicity category III

Classification: core - Guideline

This study satisfies guideline requirements (81-2) for an acute dermal toxicity study in the rat.

Reviewed by: Robert F. Fricke, Ph.D. Robert F. Fricke 10/29/92
Section IV, Tox. Branch II (H7509C)
Secondary Reviewer: Elizabeth A. Doyle, Ph.D. E.A. Doyle 4/13/92
Section IV, Tox. Branch II (H7509C)

DATA EVALUATION REPORT 009587

STUDY TYPE: Acute Inhalation - Rat (81-3)

P.C. CODE: 128724

MRID NO.: 421698-16

TEST MATERIAL: S-23031

SYNONYMS: Pentyl 2-chloro-4-fluoro-5-[(3,4,5,6-tetrahydro)phthalimido]phenoxy acetate

STUDY NUMBER: 2036

SPONSOR: Valent U.S.A. Corporation
1333 N. California Blvd
Walnut Creek, CA 94596

TESTING FACILITY: Sumitomo Chemical Co., Ltd
Biochemistry and Toxicology Laboratory
1-98, 3-Chome
Kasugade-naka, Konohana-ku
Osaka, Japan

TITLE OF REPORT: Acute Inhalation Toxicity Study of S-23031 in Rats

AUTHOR: S. Kawaguchi

REPORT ISSUED: 13 June 1990

CONCLUSIONS: Male and female rats were exposed for four hours in a whole body chamber to air only (control) or to a dust aerosol of test article at concentrations of 3,720 mg/m^3 (3.72 mg/l) or 5,940 mg/m^3 (5.94 mg/l). Transient physiological effects (irregular respiration, bradypnea, and decreased spontaneous activity) were observed from 0.5 hours into the exposure period until 2 hours into the post-exposure observation period. There were no treatment-related effects on mortality, body weight, body weight gain or pathological findings.

Male and Female: LC_{50} > 5,940 mg/m^3 (5.94 mg/l)

Toxicity Category IV

CLASSIFICATION: Core - Guideline

This study does satisfy guideline requirements (81-3) for an acute inhalation toxicity study in the rat

MATERIALS AND METHODS

Test compound: S-23031 Description: White to light brown solid Batch #: PYG-89081J Purity: 94.6% Contaminants: list in CBI appendix.

Test animals: Species: Rat Strain: Crj:CD (Sprague Dawley) Age: 6-7 weeks Weight (g): 213 - 247 (males), 157 - 182 (females) Source: Charles River Japan, Inc.

Study design: Three groups of five rats/group/sex were randomly distributed to control and treatment groups. Group I (control) received air only while Groups II and III were exposed to a dust aerosol of test article at concentrations of 3,720 (LDT) and 5,940 mg/m^3 (HDT), respectively, for four hours. Animals were observed during the exposure (0.5, 1, 2, 3 and 4 hours), immediately after the exposure (1, 2, 3 and 4 hours) and daily, thereafter, for 14 days. Animals were observed for signs of toxicity, moribundity and mortality. Rats were weighed three days before and one and six days after exposure. At the termination of the study, animals were necropsied for gross pathological and histopathological examinations.

Test atmosphere generation: The test article was pulverized into a fine powder and dispensed as a dust aerosol using a dust feeder under compressed air (2.0 kg/cm^2). The dust aerosol generated was immediately transported into the exposure chamber. The flow rate of the exhausted air was adjusted to 120 l/min. The spray rate of the dust feeder was adjusted to 1.3 g/min (LDT) or 5 g/min (HDT). Actual aerial concentrations of test article were determined at approximately 1 and 3 hours following the initiation of the exposure. The relative humidity in aerosol chamber ranged from 33 to 48 % with a temperature range of 23.7 to 25.0 °C.

Statistics: Means and standard deviations of animal body weights, chamber temperatures and relative humidities, and chamber air flows were calculated for descriptive purposes.

RESULTS AND DISCUSSION

The concentration of test article within the chamber was measured at approximately 1 and 3 hours following the initiation of the exposure, Table 1. The results indicate that the test article was in equilibrium. Further, the particle size distribution was measured; the results of a typical run are summarized in Table 2.

Due to the high density of the dust within the chamber, the animals could not be observed for signs of toxicity during the exposure. During the exposure period, irregular respiration, bradypnea and decreased spontaneous activity were observed in many rats in the treatment groups 0.5 hours after initiation of the exposure; symptoms disappeared within 2 hours after termination of the exposure. No deaths occurred in any of the groups. There were no remarkable changes attributable to

inhalation of test article on body weight, body weight gain, gross pathology or histopathology of the respiratory organs.

Table 1: Concentration of test article in chamber

Group	Time (hours)	Concentration (mg/m^3)
LDT	1	3,460
	3	3,970
	Mean	3,720
HDT	1	5,770
	3	6,110
	Mean	5,940

Table 2: Particle size distribution

Aerodynamic Diameter (μm)	Cumulative % (Run 1, Run 2)	
	LDT	HDT
11.0	95.4, 94.4	90.1, 89.8
7.00	82.8, 82.2	71.7, 70.8
4.70	53.9, 51.5	42.3, 41.8
3.30	28.3, 25.8	19.9, 19.5
2.10	10.6, 9.1	5.1, 5.6
1.10	2.2, 1.5	0.8, 0.9
0.65	0.4, 0.1	0.2, 0.3
0.43	0.2, 0.0	0.1, 0.3
5.20 - 5.23	---	MMAD
4.29 - 4.48	MMAD	---

CONCLUSIONS: Male and female rats were exposed for four hours in a whole body chamber to air only (control) or to a dust aerosol of test article at concentrations of 3,720 mg/m^3 (3.72 mg/l) or 5,940 mg/m^3 (5.94 mg/l). Transient physiological effects (irregular respiration, bradypnea, and decrease spontaneous activity) were observed from 0.5 hours into the exposure period until 2 hours into the post-exposure observation period. There were no treatment-related effects on mortality, body weight, body weight gain or pathological findings.

Male and Female: LC_{50} > 5,940 mg/m^3 (5.94 mg/l)

Toxicity category IV

Classification: core - Guideline

This study does satisfy guideline requirements (81-3) for an acute inhalation toxicity study in the rat.

Reviewed by: Robert F. Fricke, Ph.D. Robert F. Fricke 22 May 92
Section IV, Tox. Branch II (H7509C)
Secondary Reviewer: Elizabeth A. Doyle, Ph.D. E.A. Doyle
Section IV, Tox. Branch II (H7509C) 5/22/92

009587

DATA EVALUATION REPORT

STUDY TYPE: Acute Inhalation - Rat (81-3)

P.C. CODE: 128724

MRID NO.: 421698-17

TEST MATERIAL: V-23031 0.83 EC

SYNONYMS: V-23031, S-23031, Pentyl 2-chloro-4-fluoro-5-[(3,4,5,6-tetrahydro)phthalimido]phenoxy acetate

STUDY NUMBER: VLT 12/91347

SPONSOR: Valent U.S.A. Corporation
1333 N. California Blvd
Walnut Creek, CA 94596

TESTING FACILITY: Huntingdon Research Centre Ltd.
P.O. Box
Huntingdon, Cambridgeshire
PE18 6ES, England

TITLE OF REPORT: V-23031 0.83 EC Acute Inhalation Toxicity in Rats

AUTHOR: G. Jackson, C. Hardy, J. Morrow, D. Lewis and C. Gopinath

REPORT ISSUED: 3 December 1991

CONCLUSIONS: Male and female rats were placed in a whole body chamber and exposed for four hours to air only (control) or to a liquid aerosol of test compound at concentrations of 3.63, 4.90, 5.45, or 5.92 mg/l. Clinical signs observed during the exposure and immediately after consisted of respiratory effects, which were consistent with breathing an irritant vapor. The incidence of clinical signs began to decrease on observation Day 5 with complete recovery by Day 11. Of the animals which died on the study, both gross and histopathological examination revealed extensive respiratory tract damage with an increase in lung weights relative to body weight. Mortality increased with increasing dose. A transient decrease in body weight occurred immediately after exposure and lasted two days.

Male and Female: LC_{50} = 5.51 mg/l

Toxicity category IV

CLASSIFICATION: core - Guideline

This study satisfies guideline requirements (81-3) for an acute inhalation toxicity study in the rat.

MATERIALS AND METHODS

Test compound: V-23031 0.83 EC Description: clear amber liquid
Batch #: V 803L01 Purity: 94.6% Contaminants: not given.

Note: The test compound is the end use formulation consisting of 10% solution (solvent not given) of the active ingredient (V-23031). The purity and lot number of V-23031 are 94.6% and PYG-89081Y, respectively.

Test animals: Species: Rat Strain: Sprague-Dawley
Age (weeks): 6 (males) and 8 (females) Weight (g): 183 - 223 (males), 188 - 222 (females) Source: Charles River U.K., Ltd.

Study design: Animals, five rats/sex/group, were randomly assigned to control and treatment groups. Group 1 (control) received air only while Groups 2 through 6 were exposed to a liquid aerosol of test compound at concentrations of 5.92, 2.84, 3.63, 5.45, and 4.90 mg/l, respectively, for four hours. Clinical observations were taken at 0.25, 0.5, 1 hour of the equilibration period, hourly during the 4-hour exposure period and twice daily during the 14-day observation period. Rats were weighed daily, starting five days before the start of the study and through the end of the observation period. All animals were necropsied for gross pathological and histopathological examinations.

Test atmosphere generation: The test compound was pumped into the aerosol generator from syringe fitted into a constant drive syringe pump. The flow rate of the syringe pump was adjusted to deliver the appropriate amount of test compound to yield the desired final aerosol concentration. An initial flow rate of 0.6 ml/min was expected to deliver a final aerosol concentration of 5.0 mg/l. The aerosol generator was supplied by 25 l/min of dried, filtered and oil-free compressed air. The test atmosphere entered into a whole body exposure chamber through a center port at the base. The whole body chamber had a total internal volume of 120 liters and was subdivided into 10 separate animal holding compartments. The concentration of test compound in the chamber was determined at 30 min, 1 hr, 2 hr, 3 hr:5 min, and 3 hr:50 min of the exposure period. In addition to determining the concentration of test compound, the amount of active ingredient (V-23031) was determined from its concentration in the formulated product and the density of the formulation. Two additional samples, used for particle size distribution analysis, were taken at 1 hr:30 min and 3 hr:30 min. The relative humidity in aerosol chamber ranged from 35 to 65% except on two occasions where values of 66% and 72% were recorded. The temperature ranged from 25 to 26°C.

Statistics: Means and standard deviations of animal body weights, chamber temperatures and relative humidities were calculated for descriptive purposes. The LC_{50} was calculated using the probit method of Miller and Tainter (Proc. Soc. Exp. Bio. Med. **57**: 261-264, 1944).

RESULTS AND DISCUSSION

Test Atmosphere: Samples of the test atmosphere during the exposure indicated that the concentration of test compound within the chamber was in equilibrium. The mean concentrations of both the active ingredient (V-23031) and formulated product are summarized in Table 1. Particle size distribution, measured at the beginning and end of the exposure period, were comparable to each other; the mean data are summarized in Table 2.

Table 1: Mean Concentration of V-23031 and the equivalent concentration of the formulation (Summarized from Table 1 of study)

	Concentration in Air (mg/l)	
Group	V-23031	Formulation
2	0.62	5.92
3	0.30	2.84
4	0.38	3.63
5	0.57	5.45
6	0.51	4.90

Table 2: Mean Particle Size Distribution (Summarized from Table 2 of study)

	Cumulative Percent Less That Cut-off Size				
Cut-Off Size (μm)	Group 2	Group 3	Group 4	Group 5	Group 6
9.8	96.4	99.9	99.9	97.4	100
6.0	86.6	96.4	99.2	89.1	91.4
3.5	68.7	84.7	93.9	76.7	70.4
1.55	26.1	30.5	51.1	43.6	35.6
0.93	15.3	20.6	35.2	23.3	18.9
0.52	3.1	3.7	6.9	7.7	5.9
MMAD (μm)	2.4	1.8	1.3	1.8	2.0

Clinical Signs and Mortality: During the exposure period, clinical signs, consistent with contact with an irritant aerosol, were partial closing of the eyes, exaggerated breathing, reddening of the feet, and wetness around the eyes and mouth. Immediately after the exposure period, the clinical signs included exaggerated breathing, lethargy, gasping and to a lesser extent noisy breathing. Other clinical signs appearing on Day 1 of the observation period consisted of yellow staining on the head and back and around the urogenital area; brown staining was also apparent around the snout and jaws. From observation Day 5 through 11, the incidence of clinical signs decreased progressively to normal appearance and behavior.

Deaths generally occurred within the first two days of the recovery period. Gross pathological observations of the animals, which died on the study, included congested lungs and gas-filled stomachs. Histopathological examination showed extensive ulceration and erosion of the respiratory and olfactory epithelium of the nasal turbinates and larynx. To a lesser extent, the trachea and carina showed epithelial ulceration.

Rats, at terminal sacrifice, appeared normal on gross pathological examination. However, histopathological examination of the nasal turbinates showed disorganization and focal degeneration of the olfactory epithelium, hypertrophy of the respiratory epithelium; the larynx showed focal/extensive squamous metaplasia, focal hyperplasia, and mineralization of the ventral cartilage.

Body Weights: Treatment-related decreases in mean body weights of both male and female animals occurred during the first two days of the recovery period. From Day 3 to Day 14 of the recovery period to the body weight gain of the treated animals appeared to be similar to that of the controls.

Lung Weights: The lungs weights (relative to body weights) of animals which died on study were higher than control values. Animals surviving until terminal sacrifice had relative lung weights which were comparable to control values. Although means and standard deviations were determined, determination of statistical significance was not performed.

Determination of LC_{50}: Test compound at concentrations of 3.63 , 4.90, 5.45, or 5.92 mg/l produced 0%, 20%, 60% and 60% lethality, respectively. From these data an LC_{50} value of 5.51 mg/l was calculated from the combined male and female lethality data.

Conclusions: Animals, five rats/sex/group, were randomly distributed to control and treatment groups. Group 1 (control) received air only while Groups 2 through 6 were exposed to a liquid aerosol of test compound at concentrations of 5.92, 2.84, 3.63, 5.45, and 4.90 mg/l, respectively, for four hours. Clinical observations were taken at 0.25, 0.5, 1 hour of the equilibration period, hourly during the 4-hour exposure period and twice daily during the 14-day observation period. Rats were weighed daily, starting five days before the start of the study and through the end of the observation period. All animals were necropsied for gross pathological and histopathological examinations.

Male and Female: LC_{50} = 5.51 mg/l

Toxicity category IV

Classification: core - Guideline

This study satisfies guideline requirements (81-3) for an acute inhalation toxicity study in the rat.

Reviewed by: Robert F. Fricke, Ph.D. Robert F. Fricke 19 May 92
Section IV, Tox. Branch II (H7509C)
Secondary Reviewer: Elizabeth A. Doyle, Ph.D. E.A. Doyle 5/21/92
Section IV, Tox. Branch II (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: Primary Eye and Skin Irritation Tests in Rabbits (81-4 and 81-5) 009587

P.C. CODE: 128724

MRID NO.: 421698-18

TEST MATERIAL: S-23031

SYNONYMS: Pentyl 2-chloro-4-fluoro-5-[(3,4,5,6-tetrahydro)phthalimido]phenoxy acetate

STUDY NUMBER: 1693

SPONSOR: Valent U.S.A. Corporation
1333 N. California Blvd
Walnut Creek, CA 94596

TESTING FACILITY: Sumitomo Chemical Co., Ltd
Biochemistry and Toxicology Laboratory
1-98, 3-Chome
Kasugade-naka, Konohana-ku
Osaka, Japan

TITLE OF REPORT: Primary Eye and Skin Irritation Tests with S-23031 in Rabbits

AUTHOR: H. Yamada

REPORT ISSUED: 30 January 1989

CONCLUSIONS: Ocular irritation of test compound was evaluated in male and female rabbits. Application of 100 mg dry, powdered test compound produced only slight eye irritation at one hour; no irritation was present at 24 hours onward.

The test compound did not produce any skin irritation. However, the test compound was applied as a dry powder, which may not have made good contact with the skin. The test compound should have been moistened sufficiently with water or some other suitable solvent.

Toxicity category III (Eye Irritation)

Classification: core - Guideline (Eye Irritation)
Classification: core - Unacceptable (Skin Irritation)

This study satisfies guideline requirements for primary eye irritation (81-4) in rabbits but does not satisfy the guideline requirements for primary dermal irritation (81-5) in rabbits.

MATERIALS AND METHODS:

A. Test compound: S-23031 Description: White to light brown solid
Batch #: PYG-88092 Purity: 94.4% Contaminants: list in CBI appendix.

B. Test animals: Species: Rabbit Strain: New Zealand White Age: 7 weeks Weight (kg): 2.57 - 2.67 (males), 2.44 - 2.67 (females) Source: Kitayama LABES Co., Ltd., Japan

C. Study design:

1. Eye Irritation Test: Three male and three female rabbits were used in this study. Test compound (100 mg) was placed in the conjunctival sac of one eye; the other, untreated eye served as a control. The eyes were examined for the presence of irritation at 1, 24, 48 and 72 hours after treatment. Ocular lesions were graded and scored using the Draize method.

2. Skin Irritation Test: The application sites were prepared by trimming the fur off the backs of three male and three female rabbits. Two application sites were used in this study, one remained intact, while the other was lightly abraded. Dry, powdered test compound (500 mg) was spread onto a lint patch, placed on the application site, and held in place with occlusive tape. The test compound remained in contact with the skin for 4 hours, at which time the patches were removed and the application site cleaned with acetone to remove any remaining test compound. The skin was examined 4.5, 24, 48 and 72 hours after application and scored for edema and erythema using the Draize method.

RESULTS AND DISCUSSION

A. Primary Eye Irritation: Ocular lesions found after one hour included slight redness and chemosis of the conjunctival in all of the animals; slight congestion of the iris was present in one animal. From 24 hours onward no irritation was present in any of the treated eyes. A mean Draize score of 4.8 was calculated for the one hour observation.

B. Primary Skin Irritation: No skin irritation was present at any of the observation times.

C. Conclusions:

1. Primary Eye Irritation: Ocular irritation of test compound was evaluated in male and female rabbits. Application of 100 mg dry, powdered test compound produced only slight eye irritation at one hour; no irritation was present at 24 hours onward.

Toxicity category III

Classification: core - Guideline

This study satisfies guideline requirements (81-4) for a primary eye irritation study in rabbits.

2. Primary Skin Irritation: The test compound did not produce any skin irritation. However, the test compound was applied as a dry powder, which may not have made good contact with the skin. The test compound should have been moistened with water or some other suitable solvent.

Classification: core - unacceptable (can not be upgraded)

This study does not satisfy guideline requirements (81-5) for a primary skin irritation study in rabbits.

Reviewed by: Robert F. Fricke, Ph.D. Robert F. Fricke 26 May 92.
Section IV, Tox. Branch II (H7509C)
Secondary Reviewer: Elizabeth A. Doyle, Ph.D. E A. Doyle 5/27/92
Section IV, Tox. Branch II (H7509C)

DATA EVALUATION REPORT 000587

STUDY TYPE: Primary Skin Irritation in Rabbits (81-5)

P.C. CODE: 128724

MRID NO.: 421698-20

TEST MATERIAL: V-23031 0.83 EC

SYNONYMS: Pentyl 2-chloro-4-fluoro-5-[(3,4,5,6-tetrahydro)phthalimido]phenoxy acetate

STUDY NUMBER: 901125D/VLT 2/SE

SPONSOR: Valent U.S.A. Corporation
1333 N. California Blvd
Walnut Creek, CA 94596

TESTING FACILITY: Huntingdon Research Centre Ltd.
P.O. Box
Huntingdon, Cambridgeshire
PE18 6ES, England

TITLE OF REPORT: Skin Irritation to the Rabbits of V-23031 0.83 EC

AUTHOR: M. Liggett and L. McRae

REPORT ISSUED: 13 November 1991

CONCLUSIONS: Following a single four-hour exposure to test compound, skin irritation was present 30 minutes after removal of the test compound; irritation persisted, with progressively decreasing severity, through day 14. At 4 days edema and erythema were scored as slight and well-defined, respectively. The presence of blanching (slight eschar formation) through day 4 is indicative of severe skin irritation.

Toxicity category II

Classification: core - Guideline

This study satisfies guideline requirements for primary dermal irritation (81-5).

MATERIALS AND METHODS:

A. Test compound: V-23031 0.83 EC Description: clear amber liquid Batch #: V 803L01 Purity: 10% Contaminants: not given.

B. Test animals: Species: Rabbit Strain: New Zealand White Age: 12 - 13 weeks Weight (kg): 2.7 - 3.0 Source: Foxfield Farms (U.K.) Ltd., Petersfield, Hampshire, England

C. Study design: The application sites were prepared by removing the fur from the dorso-lumbar region of three male and three female rabbits. A 0.5 ml aliquot of test compound was applied to the skin, covered with a gauze pad, and occluded with elastic tape. The test compound remained in contact with the skin for 4 hours, at which time the gauze pad was removed and the application site cleaned with water to remove any remaining test compound. The skin was examined at 4.5 hr and daily, thereafter, for 14 days. Application sites were scored for edema and erythema using the Draize method.

RESULTS AND CONCLUSIONS

A. Results: The results are summarized in the attached appendix. Thirty minutes after removal of the test compound, the skin of all the rabbits showed very slight to well-defined erythema and very slight to slight edema. At 4 days slight edema and well-defined erythema with blanching were evident. Even though the erythema was scored as well-defined, the presence of blanching (slight eschar formation) increases the score to a 4 (severe skin irritation). From days 8 through 14 the severity of the skin irritation progressively decreased. The erythema was also accompanied by blanching (days 2 - 4), hyperkeratinization (days 5 - 6), and sloughing (days 8 - 14).

B. Conclusions: Following a single four-hour exposure to test compound, skin irritation was present 30 minutes after removal of the test compound; irritation persisted, with progressively decreasing severity, through day 14. At 4 days edema and erythema were scored as slight and well-defined, respectively. The presence of blanching (slight eschar formation) through day 4 is indicative of severe skin irritation.

Toxicity category II

Classification: core - Guideline

This study does satisfy guideline requirements (81-4) for a primary eye irritation study in rabbits.

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____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

✓ FIFRA registration data.

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The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

Reviewed by: Robert F. Fricke, Ph.D. Robert F. Fricke 22 May 92
Section IV, Tox. Branch II (H7509C)
Secondary Reviewer: Elizabeth A. Doyle, Ph.D.
Section IV, Tox. Branch II (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: Primary Eye Irritation in Rabbits (81-4)

P.C. CODE: 128724

MRID NO.: 421698-19

TEST MATERIAL: V-23031 0.83 EC

SYNONYMS: Pentyl 2-chloro-4-fluoro-5-[(3,4,5,6-tetrahydro)phthalimido]phenoxy acetate

STUDY NUMBER: 901150D/VLT 4/SE

SPONSOR: Valent U.S.A. Corporation
1333 N. California Blvd
Walnut Creek, CA 94596

TESTING FACILITY: Huntingdon Research Centre Ltd.
P.O. Box
Huntingdon, Cambridgeshire
PE18 6ES, England

TITLE OF REPORT: Eye Irritation to the Rabbit of V-23031 0.83 EC

AUTHOR: M. Liggett and L. McRae

REPORT ISSUED: 13 November 1991

CONCLUSIONS: The test compound produced ocular irritation (corneal opacity, conjunctival redness, chemosis and discharge) which persisted for 7 days.

Toxicity category II

Classification: core - Guideline

This study satisfies guideline requirements (81-4) for a primary eye irritation study in rabbits.

MATERIALS AND METHODS:

A. Test compound: [illegible] EC Description: Clear amber liquid Batch #: [illegible] Purity: [illegible] Contaminants: not given.

B. Test animals: Species: Rabbit Strain: New Zealand White Age: [illegible] Weight Kg: 2.8 - 3.0 Source: Froxfield Farms [illegible] Petersfield, Hampshire, England

C. Study design: Animals, one male and two females, were used in this study. A 0.1 ml aliquot of test compound was placed in the conjunctival sac of one eye; the other, untreated eye served as a control. The eyes were examined for the presence of irritation at 1 hour, 1, 2, 3, 4, 7 and 14 days after treatment. Ocular lesions were graded and scored using the Draize method.

RESULTS AND CONCLUSIONS

A. Results: The ocular reactions to test compound at one hour were limited to conjunctival reddening, chemosis and discharge; conjunctival irritation persisted through observation day 7. After 24 hours, scattered, diffuse opacity of the cornea developed, which persisted through observation day 7. No evidence of iridial irritations was observed. By 14 days no visible irritation was evident in any of the animals.

B. Conclusions: The test compound produced ocular irritation (corneal opacity, conjunctival redness, chemosis and discharge) which persisted for 7 days.

Toxicity category II

Classification: core - Guideline

This study does satisfy guideline requirements (81-4) for a primary eye irritation study in rabbits.

Reviewed by: Robert F. Fricke, Ph.D. Robert F. Fricke 1 June 92
Section IV, Tox. Branch II (H7509C)
Secondary Reviewer: Elizabeth A. Doyle, Ph.D. E.A. Doyle 6/2/92
Section IV, Tox. Branch II (H7509C)

009587

DATA EVALUATION REPORT

STUDY TYPE:	Skin Sensitization in Guinea-Pig (Buehler's Method) (81-6)
P.C. CODE:	128724
MRID NO.:	421698-21
TEST MATERIAL:	S-23031
SYNONYMS:	Pentyl 2-chloro-4-fluoro-5-[(3,4,5,6-tetrahydro)phthalimido]phenoxy acetate
STUDY NUMBER:	1880
SPONSOR:	Valent U.S.A. Corporation 1333 N. California Blvd Walnut Creek, CA 94596
TESTING FACILITY:	Sumitomo Chemical Co., Ltd Biochemistry and Toxicology Laboratory 1-98, 3-Chome Kasugade-naka, Konohana-ku Osaka, Japan
TITLE OF REPORT:	Skin Sensitization Test with S-23031 in Guinea-Pigs (Buehler's Method)
AUTHOR:	H. Yamada
REPORT ISSUED:	6 September 1989

CONCLUSIONS: Buehler's method was used to evaluate the skin sensitivity of the test compound in guinea pigs. Based on the results of the study, the test compound did not exhibit any sensitization potential. However, the test compound was apparently applied as a dry powder, which may not have made good contact with the skin. The test compound should have been moistened sufficiently with water or some other suitable solvent.

Classification: core - unacceptable (cannot be upgraded)

(Test compound was applied as a dry powder)

This study does not satisfy guideline requirements (81-6) for a dermal sensitization study in guinea pigs.

MATERIALS AND METHODS:

A. Test compound: S-23031 Description: light tan powder Batch #: PYG-88092 Purity: 94.4% Contaminants: not given.

B. Test animals: Species: Guinea pig Strain: male Hartley Age: 5 weeks Weight (g): 305 - 381 Source: Charles River Japan, Co., Ltd.

C. Study design:

1. Preliminary Dermal Irritation Study: A preliminary dermal irritation test showed that topical application of test compound did not result in the formation of either erythema or edema.

2. Main Study

a. Induction: Animals (10/group) were randomly assigned to control and treatment groups. The flank region was clipped free of fur and 0.5 g of test compound was spread onto a lint patch, applied to the skin, and held in place with an occlusive dressing for 6 hours. This same procedure was repeated weekly, for three weeks. Control animals were treated similarly, but without any test compound.

b. Challenge with Test Compound: Following a two-week resting phase, the animals in both the control and treatment groups were challenged with test compound. The procedure used for the challenge was the same as described for the induction phase. The sites were evaluated and scored 24 and 48 hours later. (Note: The study did not indicate that the challenge was done at a virgin site on the skin).

c. Positive Controls: Two positive control groups (5 animals/group) were used in this study. The procedures for induction and challenge are the same as outlined above. One group (DNCB-sensitized animals) was treated topically with a lint patch saturated with 0.5 ml of 1.0% 2,4-dinitrochlorobenzene (DNCB) in acetone. The DNCB-control animals were treated in a similar manner with acetone only. Both groups were challenged with DNCB.

RESULTS AND CONCLUSIONS

A. Results: The results of the study are presented in the attached appendix. None of the animals in the treatment, control, or DNCB-control groups showed any sensitivity at either 24 or 48 hours after the appropriate challenge. The DNCB-sensitized group responded positively.

B. Conclusions: Buehler's method was used to evaluate the skin sensitivity of the test compound in guinea pigs. Based on the results of the study, the test compound did not exhibit any sensitization potential. However, the test compound was apparently applied as a dry powder, which may not have made good contact with the skin. The test compound should have been moistened sufficiently with water or some other suitable solvent.

Studies submitted by the registrant showed marked differences between the active ingredient[1] (a dry powder) and the formulated product[2] (an emulsion) in the primary skin irritation assay. The dry powder failed to elicit any dermal effects while the formulated product produced slight to well-defined edema and erythema (with blanching).

Classification: core - unacceptable (can not be upgraded)

This study does not satisfy guideline requirements (81-6) for a dermal sensitization study in guinea pigs.

C. References

1. Primary Skin Irritation Test with S-23031 in Rabbits; Valent U.S.A. Corporation; 1693, 28 December 1989. EPA Accession No.: 421698-18.

2. Skin Irritation to Rabbit of V-23031 0.83 EC; Valent U.S.A. Corporation; 901150D/VLT 4/SE; 13 November 1991, EPA Accession No.: 421698-20.

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The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

GG9587

Reviewed by: Robert F. Fricke, Ph.D. Robert F. Fricke 1 June 92
Section IV, Tox. Branch II (H7509C)
Secondary Reviewer: Elizabeth A. Doyle, Ph.D. E.A. Doyle 6/8/92
Section IV, Tox. Branch II (H7509C)

DATA EVALUATION REPORT

STUDY TYPE:	Skin Sensitization in Guinea-Pig (Maximization Method) (81-6)
P.C. CODE:	128724
MRID NO.:	421698-22
TEST MATERIAL:	S-23031
SYNONYMS:	Pentyl 2-chloro-4-fluoro-5-[(3,4,5,6-tetrahydro)phthalimido]phenoxy acetate
STUDY NUMBER:	1698
SPONSOR:	Valent U.S.A. Corporation 1333 N. California Blvd Walnut Creek, CA 94596
TESTING FACILITY:	Sumitomo Chemical Co., Ltd Biochemistry and Toxicology Laboratory 1-98, 3-Chome Kasugade-naka, Konohana-ku Osaka, Japan
TITLE OF REPORT:	Skin Sensitization Test with S-23031 in Guinea-Pigs (Maximization Method)
AUTHOR:	T. Nakanishi
REPORT ISSUED:	6 September 1989

CONCLUSIONS: The maximization method was used to evaluate the skin sensitization potential of the test compound in guinea pigs. Based on the results of the study, the test compound was found to be an extreme skin sensitizer.

Classification: core - Guideline

This study satisfies guideline requirements (81-6) for a dermal sensitization study in guinea pigs.

MATERIALS AND METHODS:

A. Test compound: S-23031 Description: light tan powder
Batch #: PYG-88092 Purity: 94.4% Contaminants: not given.

B. Test animals: Species: Guinea pig Strain: male Hartley
Age: 6 weeks Weight (g): 343 - 441 Source: Charles River Japan, Co.

C. Study design:

1. Preliminary Dermal Irritation Study: A preliminary dermal irritation assay was carried out to determine the doses of test compound to be used in the induction and challenge phases of the skin sensitization study. For the induction phase, 0.1% solution of test compound in corn oil and a 25% mixture of test compound in petrolatum were used for intradermal injection and topical application, respectively. For the challenge phase of the assay, 25% mixture of test compound in petrolatum was used. Since the test material in petrolatum did not cause any dermal irritation, the sites were pretreated, 24 hours before application of test compound, with 0.2 g/site of 10% sodium lauryl sulfate in petrolatum.

2. Main Study

a. Induction: Animals (20/group) were randomly assigned to control and treatment groups. After clipping a 4 x 6 cm area on each animal, three duplicate injections were made on each side and consisted of: (1) 50 µl of Freund's complete adjuvant diluted 1:1 with water, (2) 50 µl of 1% test compound in corn oil, and (3) 50 µl of 1% test compound in a 1:1 dilution of Freund's complete adjuvant and water. Control animals were injected with the appropriate vehicles. One week after the intradermal injections, the guinea pigs were reshaved. Lint patches (2 x 4 cm), saturated with either 0.4 g of 25 % test compound in petrolatum (treatment group) or petrolatum only (control group), were applied to the skin, covered with occlusive tape, and held in position with adhesive tape. After a 48-hour exposure period, the dressing was removed.

b. Challenge with Test Compound: Following a two-week resting stage, the application sites were again shaved. Lint patches (2 x 2 cm) were saturated with 0.2 g of 25% test compound in petrolatum and applied to the skin of animals in the treatment and control groups. The lint patches were held in place with an occlusive dressing. After 24 hours the patches were removed and the challenge sites evaluated and scored 24 and 48 hours later.

c. Positive Controls: Two positive control groups (5 animals/group) were used in this study. One group (DNCB-sensitized animals) was treated intradermally with 0.05 µl 0.05% 2,4-dinitrochlorobenzene (DNCB) in corn oil followed one week later with dermal application of 0.4 ml of 0.5% DNCB in corn oil. A second group (DNCB control animals) received the corn oil vehicle only. Both groups of animals were subsequently challenged with 0.2 ml of 0.5% DNCB in corn oil. After 24 hours the patches were removed and the challenge sites evaluated and scored 24 and 48 hours later.

RESULTS AND CONCLUSIONS

A. Results: The results of the study are presented in attached appendix. All of the animals in the test compound-sensitized group showed dermal reactions consisting of slight to moderate erythema and edema. Control animals treated with test compound only showed no dermal reaction. In the DNCB-sensitized animals, dermal reactions consisted of moderate to severe edema and erythema. The skin of the DNCB control animals was normal after DNCB challenge.

B. Conclusions: The maximization method was used to evaluate the skin sensitization potential of the test compound in guinea pigs. Based on the results of the study, the test compound was found to be an extreme skin sensitizer.

Classification: core - Guideline

This study satisfies guideline requirements (81-6) for a dermal sensitization study in guinea pigs.

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____ The product confidential statement of formula.

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Reviewed by: Robert F. Fricke, Ph.D. R. Fricke 1 Jun 92
Section IV, Tox. Branch II (H7509C)
Secondary Reviewer: Elizabeth A. Doyle, Ph.D. E.A. Doyle 6/2/92
Section IV, Tox. Branch II (H7509C)

DATA EVALUATION REPORT

STUDY TYPE:	Skin Sensitization in Guinea-Pig (Buehler's Method) (81-6)
P.C. CODE:	128724
MRID NO.:	421698-23
TEST MATERIAL:	V-23031 0.83EC
SYNONYMS:	Pentyl 2-chloro-4-fluoro-5-[(3,4,5,6-tetrahydro)phthalimido]phenoxy acetate
STUDY NUMBER:	91722D/VLT 13/SS
SPONSOR:	Valent U.S.A. Corporation 1333 N. California Blvd Walnut Creek, CA 94596
TESTING FACILITY:	Huntingdon Research Centre Ltd. P.O. Box Huntingdon, Cambridgeshire PE18 6ES, England
TITLE OF REPORT:	Skin Sensitisation in Guinea-Pig of V-23031 0.83EC
AUTHOR:	B. Parcell and G. Healing
REPORT ISSUED:	22 October 1991

CONCLUSIONS: Buehler's method was used to evaluate the skin sensitivity of the test compound in guinea pigs. Based on the results of the study, the test compound did not exhibit any sensitization potential.

Classification: core - acceptable

This study satisfies guideline requirements (81-6) for a dermal sensitization study in guinea pigs.

MATERIALS AND METHODS:

A. Test compound: V-23031 0.83 EC (10% active ingredient) Description: clear red liquid Batch #: V 803L01 Purity: not given Contaminants: not given.

B. Test animals: Species: Guinea pig Strain: female Durkin/Hartley Age: 7 - 8 weeks Weight (g): 340 - 404 Source: D. Hall, Newchurch, Straffordshire, England

C. Study design:

1. Preliminary Dermal Irritation Study: A preliminary dermal irritation assay was carried out to determine the concentration of test compound to be used in the induction and challenge phases of the skin sensitization study. A 60% (v/v) dilution in water was chosen for induction phase while 15% and 30% dilutions in water were used for the challenge phase of the assay.

2. Main Study

a. Induction Phase: Animals were randomly assigned to control (five animals) and treatment (20 animals) groups. The left shoulder area was clipped free of fur and 0.5 ml of diluted test compound was spread onto a gauze patch, applied to the skin, and held in place with an occlusive dressing for 6 hours. This same procedure was repeated weekly, for three weeks. Control animals were treated similarly with distilled water.

b. Challenge Phase: Following a two-week resting period, the animals in both the control and treatment groups were challenged with test compound. The right flank was clipped free of fur; a gauze patch was saturated with 0.5 ml of the 30% dilution compound and placed on the anterior flank. Similarly the 15% dilution of test compound was applied to the posterior flank. Both application sites were covered with an occlusive dressing, which was removed after six hours. The sites evaluated and scored 24, 48 and 72 hours later.

c. Positive Controls: Concurrent controls were not run. However, sensitivity tests, using Buehler's method, were carried out periodically to evaluate the response of the Durkin/Hartley guinea pigs to a known sensitizer, formalin.

RESULTS AND CONCLUSIONS

A. Results: The results of the study are presented in the attached appendix. During the induction phase, well-defined erythema and slight to well-defined edema were observed in all of

the animals in the treatment group after each application of test compound. With the exception of a localized erythema in two animals in the treatment group, the responses of all the other animals were similar to controls.

B. Conclusions: Buehler's method was used to evaluate the skin sensitivity of the test compound in guinea pigs. Based on the results of the study, the test compound did not exhibit any sensitization potential.

Classification: core - acceptable

This study satisfies guideline requirements (81-6) for a dermal sensitization study in guinea pigs.

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____ The product confidential statement of formula.

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000587

Reviewed by: Robert F. Fricke, Ph.D. Robert F. Fricke 1/June 92
Section IV, Tox. Branch II (H7509C)
Secondary Reviewer: Elizabeth A. Doyle, Ph.D. E.A. Doyle 6/2/92
Section IV, Tox. Branch II (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: Skin Sensitization in Guinea-Pig (Maximization Method) (81-6)

P.C. CODE: 128724

MRID NO.: 421698-24

TEST MATERIAL: V-23031 0.83 EC

SYNONYMS: Pentyl 2-chloro-4-fluoro-5-[(3,4,5,6-tetrahydro)phthalimido]phenoxy acetate

STUDY NUMBER: 901116D/VLT 5/SS

SPONSOR: Valent U.S.A. Corporation
1333 N. California Blvd
Walnut Creek, CA 94596

TESTING FACILITY: Huntingdon Research Centre Ltd.
P.O. Box
Huntingdon, Cambridgeshire
PE18 6ES, England

TITLE OF REPORT: Skin Sensitisation in Guinea-Pig of V-23031 0.83 EC

AUTHOR: B. Parcell and S. Denton

REPORT ISSUED: 13 November 1991

CONCLUSIONS: The maximization method was used to evaluate the skin sensitization potential of the test compound in guinea pigs. Based on the results of the study, the test compound was found to be a strong sensitizer 75% (15/20) of the test animals responding positively.

Classification: core - Guideline

This study satisfies guideline requirements (81-6) for a dermal sensitization study in guinea pigs.

MATERIALS AND METHODS:

A. Test compound: V-23031 0.83 EC (10% active ingredient) Description: clear amber liquid Batch #: V 803L01 Purity: not given Contaminants: not given.

B. Test animals: Species: Guinea pig Strain: female Durkin/Hartley Age: 5 - 7 weeks Weight (g): 432 - 534 Source: D. Hall, Newchurch, Straffordshire, England

C. Study design:

1. Preliminary Dermal Irritation Study: A preliminary dermal irritation assay was carried out to determine the doses of test compound to be used in the induction and challenge phases of the skin sensitization study. For the induction phase, 0.1% and 15% dilutions (v/v in water) were used for intradermal injection and topical application, respectively. For the challenge phase of the assay, test compound was applied topically at dilutions of 10% and 5% (v/v) in water.

2. Main Study

a. Induction: Twenty animals were randomly assigned to the treatment group. On each animal six intradermal injection sites were prepared by clipping a 4 x 6 cm area on the back. Three duplicate injections were made on each side and consisted of: (1) 100 µl of Freund's complete adjuvant diluted 1:1 with water, (2) 100 µl of test compound, 0.1% v/v in water, and (3) 100 µl of test compound, 0.1% v/v in a 1:1 dilution of Freund's adjuvant and water. Ten negative control animals were injected intradermally with 100 µl of the appropriate vehicle without any test compound. One week after the intradermal injections, the guinea pigs were reshaved. Approximately 400 µl of the 15% mixture of test compound or vehicle (control) was applied to a 2 x 4 cm piece of Whatman No. 3 filter paper. The filter paper was applied to the skin, covered with occlusive tape, and held in position with adhesive tape. After a 48-hour exposure period, the dressing was removed.

b. Challenge with Test Compound: Following a two-week resting stage, the application sites were reshaved. Two filter paper patches (2 x 2 cm) were saturated with approximately 200 µl of 10% (anterior challenge site) and 5% (posterior challenge site) dilutions of the test compound were applied to the skin, covered with an occlusive tape and secured in place with adhesive tape. After 24 hours the patches were removed and the challenge sites evaluated and scored at 24, 48 and 72 hours later

c. Positive Controls: Concurrent controls were not run. However, sensitivity tests were carried out periodically to evaluate the response of the Durkin/Hartley guinea pigs to a known sensitizer, formalin.

RESULTS AND CONCLUSIONS

A. Results

1. The results of the study are presented in attached appendix. The majority of the control animals showed no erythema or edema; one control animal showed very slight erythema on the anterior (10% test compound) challenge site. Fifteen (75%) of the test animals showed dermal reactions consisting of slight to well-defined erythema and edema. The remaining test animals either showed no or very slight evidence of skin sensitization.

B. Conclusions: The maximization method was used to evaluate the skin sensitization potential of the test compound in guinea pigs. Based on the results of the study, the test compound was found to be a strong sensitizer with 75% (15/20) of the test animals responding positively.

Classification: core - Guideline

This study satisfies guideline requirements (81-6) for a dermal sensitization study in guinea pigs.

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Reviewed by: Robert F. Fricke, Ph.D. Robert Fricke 7/1/92
Section IV, Tox. Branch II (H7509C)
Secondary Reviewer: Elizabeth A. Doyle, Ph.D. E.A. Doyle 7/1/92
Section IV, Tox. Branch II (H7509C)

DATA EVALUATION REPORT

G092567

STUDY TYPE: 90 day Oral - Rats (82-1)

P.C. CODE: 128724

MRID NO.: 421698-26 and 423108-01 (Addendum)

TEST MATERIAL: S-23031

SYNONYMS: Pentyl 2-chloro-4-fluoro-5-[(3,4,5,6-tetrahydro)phthalimido] phenoxyacetate

STUDY NUMBER: 1632

SPONSOR: Valent U.S.A. Corporation
1333 N. California Blvd
Walnut Creek, CA 94596

TESTING FACILITY: Sumitomo Chemical Co., Ltd
Biochemistry and Toxicology Laboratory
1-98, 3-Chome
Kasugade-naka, Konohana-ku
Osaka, Japan

TITLE OF REPORT: Three-Month Subacute Toxicity Study of S-23031 by Dietary Administration in Rats

AUTHOR: Y. Yoshida

REPORT ISSUED: 9 November 1990

CONCLUSIONS: Male and female rats were given test article daily, for 13 weeks, at dosages of 0, 100, 1000, 10000 or 20000 ppm (equivalent to 0, 6.6, 67.0, 664 or 1359 mg/kg/day for males and 0, 7.4, 73.8, 726 or 1574 mg/kg/day for females, respectively).

	MALE	FEMALE
NOEL	10000 ppm (MDT2)	100 ppm (LDT)
LOEL	20000 ppm (HDT)	1000 ppm (MDT1)

Classification: core - Guideline

LOEL is based on increased relative liver weights (male) and increased cholinesterase activity (female).

This study does satisfy guideline requirements (82-1) for a 90-day feeding study in rats.

A. MATERIALS:

1. Test compound: S-23031, technical Description: brown granule or powder Batch #: PYG-88092 (granule) and PYG-88092-M (powder) Purity: 94.4% (granule) and 94.7% (powder) Contaminants: list in CBI appendix.

2. Test animals: Species: Rat Strain: Crj:CD(SD) Age: 5 weeks Weight (g): 137 - 166 (males), 115 - 140 (females) Source: Charles River Japan, Inc.

B. STUDY DESIGN:

1. Animal assignment: Animals were assigned randomly to main study test groups as shown in Table 1. An interim sacrifice was carried out after 4 weeks.

Table 1: Animal Assignment to Study Groups

Test Group		Dose in Diet (ppm)	Interim Study (4 weeks)		Main Study (13 weeks)	
			Male	Female	Male	Female
Control	(CON)	0	6	6	12	12
Low	(LDT)	100	6	6	12	12
Mid1	(MDT1)	1000	6	6	12	12
Mid2	(MDT2)	10000	6	6	12	12
High	(HDT)	20000	6	6	12	12

2. Diet preparation: Granulated and powdered forms of test article were used in the study; both forms were equal in quality (synthesized at the same time and almost the same purity and composition of impurities). Because of the two different forms, the test diets were prepared using two different methods. For granular test article, an appropriate amount was ground with a mortar and pestle and added to the basal diet to form a premix. Premixes were prepared for the 100, 1000 and 20000 ppm test diets. The premixes were blended with basal diet to the final desired concentration. The 10000 ppm test diet was prepared by making a two-fold dilution of the 20000 ppm diet with the basal diet. For preparation of the test diets using the powdered-form of the test article, a premix (one for each dose group) was made using an appropriate amount of test article and thoroughly mixing it with the basal diet. Basal diet was added to the different premixes to form the correct dietary concentration of test article. Analysis of samples, taken from the top, middle and bottom of the prepared diets, showed that the test article was homogeneously distributed (coefficient of variation 0.5 to 2.1%) and within 91 to 102% of nominal concentration.

3. Animals received diet (CRF-1, Oriental Yeast Co., Ltd., Tokyo, Japan) and water ad libitum. For determination of water consumption, water was administered using water bottles.

4. Statistics: A one-way analysis of variance (ANOVA) was performed for body weight, food consumption, water consumption, hematology, blood chemistry and organ weight. If a significant ANOVA result was found, pair-wise comparisons were carried out using the Least Significant Difference test. For urinalysis, Scheffe's mean rank test was used to test for significant difference from the control group. Significant data were further analyzed using the Kruskal-Wallis analysis of ranks. No statistical procedure was presented for the analysis of incidence data for gross pathological or histopathological observations.

5. Quality assurance was documented by signed and dated GLP and quality assurance statements.

6. The sponsor applied the criteria of 40 CFR 158.34 for flagging studies for potential adverse effects to the results of this study. This study neither meets nor exceeds any of the applicable criteria.

C. METHODS AND RESULTS:

1. Observations: Animals were inspected twice (weekdays) or once (weekends, holidays) daily for signs of toxicity, moribundity and mortality.

a. Toxicity: The only clinical observations attributable to the administration of the test article were occasional urine incontinence and stains of the fur observed in animals in the 20000 ppm groups.

b. Mortality (survival): All animals survived until the scheduled sacrifice.

2. Body weight: Animals were weighed at the start of the study, on days 5 and 8 and weekly, thereafter.

Results: During the course of the study no significant, treatment-related differences in body weights were noted in either the male or female animals.

3. Food and water consumption and compound intake: Food and water consumption were measured weekly during a consecutive 48 hour period.

a. Food consumption results: Slight, but statistically significant, increases in food consumption were noted in male and female animals in the 20000 ppm group. The data are summarized in the

following table:

Table 2: Food Consumption Data (g/animal/day)

Sex	Day	CON	LDT	MDT1	MDT2	HDT
Male	21	23	23	24	24	25**
Female	35	17	19	19	17	21**
	56	15	17	17	16	19**
	63	16	18	17	16	20**
	84	16	17	17	16	18**
	91	15	17*	17	16	18**

* $p < 0.05$, ** $p < 0.01$

b. Food efficiency results:

Table 3: Relative Food Consumption (g/kg body weight/day)

Sex	Day	CON	LDT	MDT1	MDT2	HDT
Male	21	77.0	76.7	78.6	78.9	81.6**
	77	48.3	50.5	51.9*	52.0*	52.5**
	84	46.8	49.0*	47.9	49.0*	47.9
Female	21	80.7	83.3	84.7	88.1**	87.0*
	35	76.8	81.0	79.9	75.4	89.4**

* $p < 0.05$, ** $p < 0.01$

c. Compound intake: The mean compound intake is summarized in Table 4, below.

Table 4: Compound Intake

Dose in Diet (ppm)	Compound Intake (mg/kg/day) Male	Female
0	0.0	0
100	6.6	7.4
1000	67.0	73.8
10000	664	726
20000	1359	1574

c. Water consumption: The mean and relative water consumption was measured weekly during the course of the study. Significant results are given in Table 5 and 6, below.

Table 4: Mean Water Consumption (ml/animal/day)

Sex	Day	CON	LDT	MDT1	MDT2	HDT
Male	14	35	33	32	38	40*
	21	37	34	35	43*	44**
Female	7	26	25	26	29	33**
	14	27	26	29	31	35**
	21	27	28	31	33*	37**
	28	31	32	34	36	39**
	35	36	35	37	37	48**

* $p < 0.05$, ** $p < 0.01$

Table 5: Relative Water Consumption (ml/kg body weight/day)

Sex	Day	CON	LDT	MDT1	MDT2	HDT
Male	7	162.1	159.4	153.1	178.5*	176.6*
	14	139.9	131.4	125.3	150.2*	158.7*
	21	123.9	116.4	116.0	141.8*	146.2**
Female	7	177.3	170.3	181.5	195.7	230.4**
	14	149.4	148.3	159.0	173.6	198.2**
	21	138.0	143.3	159.0	169.3**	186.7**

* $p < 0.05$, ** $p < 0.01$

4. Ophthalmological examinations: Examinations were performed on all animals at weeks 4 and 12. No treatment-related eye lesions were observed.

5. Urinalysis: Urinalysis was performed at weeks 4 and 12. The checked (X) parameters were examined.

	Volume	X	Glucose
	Specific gravity	X	Ketone Bodies
X	Protein		Bile Pigments
X	Appearance	X	Urobilirubin
X	Sediment	X	Total Bilirubin
X	pH	X	Occult Blood

Results: No treatment-related effects were noted in any of the parameters.

6. Hematology and Clinical Chemistry: Hematology and clinical chemistry analyses were performed at the scheduled sacrifice on all animals after an overnight fast. The checked (X) parameters were examined.

a. Hematology

X	Hematocrit (HCT)		Prothrombin time
X	Hemoglobin (HGB)	X	Leukocyte differential count
X	Leukocyte count (WBC)	X	Mean corpuscular HGB (MCH)
X	Erythrocyte count (RBC)	X	Mean corpuscular HGB conc.(MCHC)
X	Platelet count	X	Mean corpuscular volume (MCV)
	Bone marrow		

Results: Hematology results of the 4-week interim sacrifice revealed several significant findings in the male rats. Animals in the 100 and 1000 ppm groups showed decreases in both the neutrophil count and percent neutrophils and an increase in the percent lymphocytes. Since these observations were not dose-related, they were not treatment-related effects. At the conclusion of the study, hematology results showed statistically significant, but not toxicologically significant, decreases in both MHC and MCHC of the male rats. Female rats showed slight decreases in the platelet counts, with values of 1258, 1051, 1137, and 1149 x $10^3/\mu l$ at 0, 100, 1000 and 20000 ppm, respectively. Again, these findings are not of toxicological significance.

b. Clinical Chemistry

Electrolytes		Other	
X	Calcium	X	Albumin
X	Chloride	X	Blood creatinine
	Magnesium	X	Blood urea nitrogen
X	Phosphorous	X	Total cholesterol
X	Potassium	X	Globulins
X	Sodium	X	Glucose
Enzymes		X	Total Bilirubin
X	Alkaline phosphatase	X	Triglycerides
X	Cholinesterase	X	Total Protein
X	Creatinine phosphokinase	X	Phospholipid
X	Lactic acid dehydrogenase	X	Direct Bilirubin
X	Leucine Aminopeptidase	X	Serum Protein Fractionation
X	γ-Glutamyl transpeptidase		
X	Serum alanine aminotransferase (SGPT/ALT)		
X	Serum aspartate aminotransferase (SGOT/AST)		

Results: At the 4-week interim sacrifice, several significant findings were noted. However, the magnitude of the changes was not great enough to be of toxicological significance. At the terminal sacrifice leucine aminopeptidase activity was significantly decreased in the males and cholinesterase activity was significantly increased in the females (Table 6).

Table 6: Terminal Sacrifice Clinical Chemistry Results

Parameter (Sex)	Enzyme Activity (U/l) CON	LDT	MDT1	MDT2	HDT
Leucine Aminopeptidase (Male)	71	71	70	66*	64**
Cholinesterase (Female)	3325	3393	4132*	4198*	4700**

* $p \leq 0.05$, ** $p \leq 0.01$

7. Sacrifice and Pathology: Detailed pathological examination was performed on male and female animals in the control and treatment groups. The checked (X) tissues were collected for histological examination; the checked (XX) organs were also weighed.

Digestive system	Cardiovas./Hematol	Neurologic
X Tongue	X Aorta	XX Brain
Salivary glands	XX Heart	X Periph. nerve
X Esophagus	X Bone marrow	X Spinal cord
X Stomach	X Lymph nodes	XX Pituitary
X Duodenum	XX Spleen	X Eyes
X Jejunum	XX Thymus	Glandular
X Ileum	Urogenital	XX Adrenals
X Cecum	XX Kidneys	Lacrimal gland
X Colon	X Urinary bladder	X Mammary gland
X Rectum	XX Testes	X Parathyroids
XX Liver	X Epididymides	XX Thyroids
Gall bladder	XX Prostate	Other
X Pancreas	X Seminal vesicle	X Bone
Respiratory	XX Ovaries	X Skeletal muscle
X Trachea	X Uterus	X Skin
X Lungs	X Vagina	X Gross lesions
Nasal Passages	Cervix	X Harderian glands
		X Submandibular glands

a. Organ Weights: Significant findings for the absolute and relative organ weights at the interim sacrifice (28 days) and the terminal sacrifice (91 days) are sh[illegible]n in Table 7, below. At 28 days females showed no t[illegible]ment-related changes in either the absolute or [illegible]lative organ weights, while the males showed an increase in both the absolute and relative liver weights. At 91 days both the males and females showed an increase in the absolute liver weights, relative liver weight and relative kidney weights.

Table 5: Absolute (g) and Relative (g/kg body wt) Organ Weights

Observation	Sex	Day	CON	LDT	MDT1	MDT2	HDT
Absolute Liver Wt	♂	28	9.34	9.14	9.25	9.75	11.04
	"	91	12.26	11.77	12.83	13.14	15.13**
	♀	91	6.55	6.86	6.43	6.81	7.27**
Relative Liver Wt	♂	28	2.89	3.03	2.99	3.24	3.59**
	"	91	2.59	2.55	2.68	2.83	3.24**
	♀	91	2.52	2.53	2.43	2.63	2.73**
Relative Kidney Wt	♂	91	0.66	0.66	0.69	0.73**	0.76**
	♀	91	0.71	0.70	0.69	0.77**	0.75**

* $p < 0.05$, ** $p < 0.01$

b. Gross Pathology: The gross pathology was carried out during the interim and terminal sacrifices. In the males the only significant findings were pale and enlarged livers in the 20000 ppm group; in the females no treatment-related effects were observed.

c. Microscopic Pathology

1) Non-neoplastic: Although histopathological lesions were observed in both the control and treatment groups, none was considered to be treatment-related.

2) Neoplastic: Not noted

D. DISCUSSION: Male and female rats were given test article daily, for 13 weeks, at dosages of 0, 100, 1000, 10000 or 20000 ppm (equivalent to 0, 6.6, 67.0, 664 or 1359 mg/kg/day for males and 0, 7.4, 73.8, 726 or 1574 mg/kg/day for females, respectively).

	MALE	FEMALE
NOEL	10000 ppm (MDT2)	100 ppm (LDT)
LOEL	20000 ppm (HDT)	1000 ppm (MDT1)

Classification: core - Guideline

LOEL is based on increased relative liver weights (male) and increased cholinesterase activity (female).

This study does satisfy guideline requirements (82-1) for a 90-day feeding study in rats.

Reviewed by: Robert F. Fricke, Ph.D. Robert F. Fricke 30 June 92
Section IV, Tox. Branch II (H7509C)
Secondary Reviewer: Elizabeth A. Doyle, Ph.D. E.A. Doyle 6/30/92
Section IV, Tox. Branch II (H7509C)

DATA EVALUATION REPORT

009587

STUDY TYPE: 90 day Oral - Dogs (82-1)

P.C. CODE: 128724

MRID NO.: 421698-27 and 423108-02 (Addendum)

TEST MATERIAL: S-23031

SYNONYMS: Pentyl 2-chloro-4-fluoro-5-[(3,4,5,6-tetrahydro)phthalimido] phenoxyacetate

STUDY NUMBER: 1823

SPONSOR: Valent U.S.A. Corporation
1333 N. California Blvd
Walnut Creek, CA 94596

TESTING FACILITY: Sumitomo Chemical Co., Ltd
Environmental Health Science Laboratory
1-98, 3-Chome
Kasugade-naka, Konohana-ku
Osaka, Japan

TITLE OF REPORT: Three-Month Oral Toxicity Study of S-23031 in Dogs

AUTHOR: M. Nakano

REPORT ISSUED: 30 January 1991

CONCLUSIONS: Male and female dogs were given test article daily, for 13 weeks, at oral dosages of 0, 10, 100 or 1000 mg/kg/day. The only significant finding in male animals was slight to mild vacuolation of the cortical tubular epithelial cells in the kidneys in the 1000 mg/kg/day group. In females, a prolongation of the APTT was observed in the 100 and 1000 ppm groups.

	MALE	FEMALE
NOEL	100 mg/kg/day	10 mg/kg/day
LOEL	1000 mg/kg/day	100 mg/kg/day

LOEL based on lipid vacuoles in the kidney (males) and prolonged APTT (females).

CLASSIFICATION: core - Guideline

This study satisfies guideline requirements (82-1) for a 90-day feeding study in dogs.

A. MATERIALS:

1. Test compound: S-23031, technical Description: brown powder Batch #: PYG-88092-M Purity: 94.7% Contaminants: list in CBI appendix.

2. Test animals: Species: Dog Strain: Beagle Age: 6 - 7 months Weight (kg): 7.4 - 11.2 (males), 7.6 - 10.1 (females) Source: White Eagle Laboratories (U.S.A.) via Kasho Co. Ltd.

B. STUDY DESIGN:

1. Animal assignment: Animals were assigned randomly to main study test groups as shown in Table 1.

Table 1: Animal Assignment to Study Groups

Test Group		Dosage (mg/kg/day)	Main Study (13 weeks) Male	Female
Control	(CON)	0	4	4
Low	(LDT)	10	4	4
Mid	(MDT)	100	4	4
High	(HDT)	1000	4	4

2. Dose preparation: Based on the most recent body weight of the animal, the appropriate amount of test article was weighed and placed in hard gelatin capsules (1/8 oz J & I type, Chemical and Pharmaceutical Industry Co.). Control animals received capsules only.

3. Each animal was given 300 g of solid dog food (Lab Diet II, Purina-Taiyo Pet-Food Co., Ltd) once a day (about 2 p.m.) and allowed to eat freely until about 9 a.m. the next morning. Water was provided ad libitum.

4. Statistics: Statistical procedures are summarized in the addendum to the main study. Data were initially analyzed for homogeneity of variances using Bartlett's test. Homogeneous data were analyzed using analysis of variance (ANOVA). Data sets yielding a significant ANOVA result were further analyzed using either Dunnett's test (equal N) or the Scheffé test (unequal N). If data were not homogeneous they were analyzed using Kruskal-Wallis' nonparametric ANOVA. A significant result was further analyzed using either Dunnett's test or the Scheffé test.

5. Quality assurance was documented by signed and dated GLP and quality assurance statements.

6. The sponsor applied the criteria of 40 CFR 158.34 for

flagging studies for potential adverse effects to the results of this study. This study neither meets nor exceeds any of the applicable criteria.

C. METHODS AND RESULTS:

1. Observations: On weekdays animals were inspected every two to three hours after the administration of the test article; on holidays animals were inspected at least three times.

a. Toxicity: Emesis, loose feces, mucous feces and/or diarrhea were sometimes observed. The frequency of these observations in the treatment groups was not different from that in the control group, indicating no treatment-related effect.

b. Mortality (survival): All animals survived until the scheduled sacrifice.

2. Body weight: Animals were weighed weekly during the two week acclimation period and during the study.

Results: There were no treatment-related effects on the body weights of either the male or female dogs. No significant differences between the treatment and the control groups were noted.

3. Food consumption: Food consumption was measured daily. Food efficiency was calculated from the total food consumption per week and body weight gain per week.

a. Food consumption results: No statistically significant differences in food consumption were observed between the control and treated groups.

b. Food efficiency results: No statistically significant differences in food efficiency were observed between the control and treated groups.

4. Ophthalmological examinations: Examinations were performed on all animals at weeks 0, 5 and 11. No treatment-related eye lesions were observed.

5. Fecal Examination: Fecal samples were collected during the same 24-hour period in which urine was collected. Fecal samples were taken at weeks 0, 6 and 12 of study and examined for fecal occult blood. No abnormalities were noted in any animal.

6. Liver Function Test: The BSP retention test was performed on weeks 0, 6 and 13 of the study. There was no significant treatment-related effect on the percent retention of BSP.

7. Renal Function Test: The PAH retention test was performed on weeks 0, 5 and 11 of study. The renal clearance of PAH was not affected by treatment.

8. Electrocardiogram: Electrocardiograms were recorded via standard limb lead II without anesthesia on weeks 0, 5 and 13 of study. No significant differences were noted on P wave amplitude, R wave amplitude, XPR interval, XQRS interval and QT interval

9. Hematology: Hematology was performed at the scheduled sacrifice on all animals after an overnight fast. The checked (X) parameters were examined.

X	Hematocrit (HCT)	X	Prothrombin time
X	Hemoglobin (HGB)	X	Leukocyte differential count
X	Leukocyte count (WBC)	X	Mean corpuscular HGB (MCH)
X	Erythrocyte count (RBC)	X	Mean corpuscular HGB conc.(MCHC)
X	Platelet count	X	Mean corpuscular volume (MCV)
X	Bone marrow	X	Reticulocyte count
X	Activated partial thromboplastin time (APTT)		

Results: Hematological evaluation of control and treated male dogs did not reveal any pronounced treatment-related effects. In females, however, a significant prolongation of the APTT was observed in the 100 and 1000 ppm groups (Table 2).

Table 2: Activated partial thromboplastin time (sec) in Female Dogs

Week	CON	LDT	MDT	HDT
0	22.6	22.2	21.7	23.3
4	25.1	25.2	26.2**	30.6**
8	22.1	22.2	24.1*	26.2**
12	22.7	22.5	22.3	28.2**

* $p \leq 0.05$, ** $p \leq 0.01$

10. Urinalysis: Urinalysis was performed at weeks 0, 6 and 12. The checked (X) parameters were examined.

X	Volume	X	Glucose
X	Specific gravity	X	Ketone Bodies
X	Protein		Bile Pigments
X	Appearance	X	Urobilirubin
X	Sediment	X	Total Bilirubin
X	pH	X	Occult Blood

Results: No treatment-related effects were noted in any of the parameters in any of the groups.

11. Clinical Chemistry: Clinical chemistry analyses were performed at the scheduled sacrifice on all animals after an overnight fast. The checked (X) parameters were examined.

Electrolytes	Other
X Calcium	X Albumin
X Chloride	X Blood creatinine
Magnesium	X Blood urea nitrogen
X Phosphorous	X Total cholesterol
X Potassium	X Globulins
X Sodium	X Glucose
Enzymes	X Total bilirubin
X Alkaline phosphatase	X Triglycerides
Cholinesterase	X Total protein
X Creatinine phosphokinase	X Phospholipid
X Lactic acid dehydrogenase	X Uric acid
X γ-Glutamyl transpeptidase	X Serum protein fract.

X Serum aspartate aminotransferase (SGOT/AST)
X Serum alanine aminotransferase (SGPT/ALT)

Results: No significant treatment-related changes were noted in any of the clinical chemistry parameters measured.

12. Sacrifice and Pathology: Detailed pathological examination was performed on male and female animals in the control and treatment groups. The checked (X) tissues were collected for histological examination; the checked (XX) organs were also weighed.

Digestive system	Cardiovas./Hematol	Neurologic
X Tongue	X Aorta	XX Brain
Salivary glands	XX Heart	X Periph. nerve
X Esophagus	X Bone marrow	X Spinal cord
X Stomach	X Lymph nodes	XX Pituitary
X Duodenum	XX Spleen	X Eyes
X Jejunum	XX Thymus	Glandular
X Ileum	Urogenital	XX Adrenals
X Cecum	XX Kidneys	Lacrimal gland
X Colon	X Urinary bladder	X Mammary gland
X Rectum	XX Testes	X Parathyroids
XX Liver	X Epididymides	XX Thyroids
XX Gall bladder	XX Prostate	Other
XX Pancreas	X Seminal vesicle	X Bone marrow
Respiratory	XX Ovaries	X Skeletal muscle
X Trachea	XX Uterus	X Skin
XX Lungs	X Vagina	X Gross lesions
Nasal Passages	Cervix	XX Mandibular glands
X Larynx		X Parotid glands

a. Organ Weights: Compared to the control group, no significant differences in either the absolute or relative organ weights were observed in any of the treatment groups.

b. Gross Pathology: Macroscopic examination of tissues taken at the terminal sacrifice did not reveal any treatment-related abnormalities.

c. Histopathology

1) Non-neoplastic: The only treatment-related microscopic findings that were slight to mild vacuolation of the cortical tubular epithelial cells in the kidneys of all the male animals in the 1000 mg/kg/day group. The vacuoles were lipid drops, as demonstrated by positive staining with Oil Red O. Electron microscopic examination also showed an increase in lipid drops in the proximal tubular epithelial cells.

2) Neoplastic: Not noted

D. DISCUSSION: Male and female dogs were given test article daily, for 13 weeks, at oral dosages of 0, 10, 100 or 1000 mg/kg/day. The only significant finding in male animals was slight to mild vacuolation of the cortical tubular epithelial cells in the kidneys in the 1000 mg/kg/day group. In females, a prolongation of the APTT was observed in the 100 and 1000 ppm groups.

	MALE	FEMALE
NOEL	100 mg/kg/day	10 mg/kg/day
LOEL	1000 mg/kg/day	100 mg/kg/day

LOEL based on lipid vacuoles in the kidney (males) and prolonged APTT (females)

Classification: core - Guideline

This study satisfies guideline requirements (82-1) for a 90-day feeding study in dogs.

Reviewed by: Robert F. Fricke, Ph.D. Robert F. Fricke 10 Apr 92
Section IV, Tox. Branch II (H7509C)
Secondary Reviewer: Elizabeth A. Doyle, Ph.D. E. A. Doyle 4/13/92
Section IV, Tox. Branch II (H7509C)
009587

DATA EVALUATION RECORD

STUDY TYPE: Range-Finding Oral Toxicity - Rabbit

P.C. CODE: 128724

MRID NO.: 421698-28

TEST MATERIAL: S-23031

SYNONYMS: Pentyl 2-chloro-4-fluoro-5-[(3,4,5,6-tetrahydro) phthalimido] phenoxyacetate

STUDY NUMBER: HLA 343-219

SPONSOR: Sumitomo Chemical Co., Ltd
Kitahama, 4-Chome 5-33, Chuo-Ku
Osaka, Japan

TESTING FACILITY: Hazleton Laboratories America, Inc.
9200 Leesburg Turnpike
Vienna, VA 22182

TITLE OF REPORT: 5-Day Oral Toxicity Study in Female Rabbits with S-23031

AUTHOR: S.L. Morseth

REPORT ISSUED: 24 May 1990

CONCLUSIONS: This range-finding study evaluated the oral toxicity of test article at dosages of 0, 300, 500, 1000 and 1500 mg/kg/day in female rabbits. The results of this study will be used as a basis in defining the dosages to be used in subsequent developmental toxicity studies. From the data provided, treatment-related toxicity was present in the animals in the 1500 mg/kg/day group. The toxic effects consisted of decreased body weight gain and decreased food consumption.

NOEL = 1000 mg/kg/day

LOEL = 1500 mg/kg/day

LOEL based on decreased body weight gain and decreased food consumption.

CLASSIFICATION: Core - Supplementary

This is not a guideline study.

A. MATERIALS

1. Test compound: S-23031, technical Description: brown powder Batch #: PYG-88092-M Purity: 94.4% Contaminants: list in CBI appendix.

2. Dose Preparation: Test article was suspended in 0.5% methylcellulose (Lot No. 14F-0545, Sigma Chemical Co., St. Louis, MO) in water (Polar Distilled Water, Lot No. 1223902, All Pure Spring Water, Inc. Oakland, MD). Samples from the top, middle and bottom of all the dosing solutions were analyzed and found to be homogeneous (relative standard deviations of 1.5 to 2.6%) and within 96.6 and 101% of the nominal concentration.

3. Test Animal: Species: Rabbit Strain: Female, Hra:(NZW)SPF Source: Hazleton Research Products, Inc, Denver, PA Age: 4 months Weight: 2482 - 3258 g.

4. Animals received Certified Rabbit Chow (Purina, #5322) and water were provided ad libitum.

B. STUDY DESIGN:

1. Group Arrangement: The animals were randomly assigned to the following test groups:

Table 1: Animal Assignment to Study Groups

Test Group		Dosage (mg/kg/day)	Number Assigned
Control	(CON)	0	5
Low	(LDT)	300	5
Middle 1	(MDT1)	500	5
Middle 2	(MDT2)	1000	5
High	(HDT)	1500	5

2. Dosing: Concentrations of test article in the dosing solutions varied with dose (dose volume was kept constant at 5.0 ml/kg).

3. Statistics: Data were analyzed for homogeneity of variances using Levene's Test. If found to be homogeneous, an analysis of variance (ANOVA) was performed. If the data were heterogeneous, a rank transformation was performed. If the transformed data were homogeneous, ANOVA was carried out. A significant ANOVA result was followed by Dunnett's Test to determine significant differences between the control and treated groups.

4. Quality assurance was documented by signed and dated GLP and quality assurance statements.

5. The sponsor applied the criteria of 40 CFR 158.34 for flagging studies for potential adverse effects to the results of this study. This study neither meets nor exceeds any of the applicable criteria.

C. METHODS AND RESULTS

1. Observations: Animals were inspected twice daily for signs of toxicity, moribundity and mortality.

a. Toxicity: No remarkable clinical observations were noted.

b. Mortality (survival): All animals survived until the scheduled sacrifice.

2. Body weight: Animals were weighed daily and the mean body weight and the body weight changes determined. Due to malfunctioning water delivery system, animals in 0, 300 and 500 mg/kg/day groups were without water for the first two days of the study. Because of dehydration in these groups, the mean body weights (Table 2) and the body weight changes (Table 3) during this time period could not be attributed to the administration of test article. However, the body weight gain for day 2 to 3 and 3 to 4 were significantly lower in the 1500 mg/kg/day group, indicating a treatment-related effect.

3. Food consumption: Food consumption was measured daily and is summarized in Table 4, below. Due to the watering problem noted above, the food consumption data for the first two days of the study are inaccurate. During the latter part of the study, however, the mean food consumption of the animals in the 1500 mg/kg/day group was significantly lower than the control.

4. Sacrifice and Pathology: Pathological examination was performed on the animals in the control and treatment groups. A list of the organs examined was not provided in the study. The study indicates that no remarkable observation were seen in any animal.

D. DISCUSSION: This range-finding study evaluated the oral toxicity of test article at dosages of 0, 300, 500, 1000 and 1500 mg/kg/day in female rabbits. The results of this study will be used as a basis in defining the dosages to be used in subsequent developmental toxicity studies. From the data provided, treatment-related toxicity was present in the animals in the 1500 mg/kg/day group. The toxic effects consisted of decreased body weight gain and decreased food consumption.

NOEL = 1000 mg/kg/day

LOEL = 1500 mg/kg/day

LOEL based on decreased body weight gain and decreased food consumption.

CLASSIFICATION: Core - Supplementary

This is not a guideline study.

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Reviewed by: Robert F. Fricke, Ph.D. Robert F. Fricke 11 May 92
Section IV, Tox. Branch II (H7509C)
Secondary Reviewer: Elizabeth A. Doyle, Ph.D. E.A. Doyle 5/5/92
Section IV, Tox. Branch II (H7509C)

DATA EVALUATION RECORD 009587

STUDY TYPE: Range Finder - Teratology - Developmental Toxicity - Rabbit

P.C. CODE: 128724

MRID NO.: 421698-29

TEST MATERIAL: S-23031

SYNONYMS: Pentyl 2-chloro-4-fluoro-5-[(3,4,5,6-tetrahydro) phthalimido] phenoxyacetate

STUDY NUMBER: HLA 343-220

SPONSOR: Sumitomo Chemical Co., Ltd
Kitahama, 4-Chome 5-33, Chuo-Ku
Osaka, Japan

TESTING FACILITY: Hazleton Laboratories America, Inc.
9200 Leesburg Turnpike
Vienna, VA 22182

TITLE OF REPORT: Range-Finding Rabbit Teratology Study with S-23031

AUTHOR: S.L. Morseth

REPORT ISSUED: 24 May 1990

CONCLUSIONS: The developmental effects of S-23031 on rabbits was evaluated in a range-finding study in which the animals were dosed at 0, 300, 500, 1000 or 1500 mg/kg/day throughout the organogenesis period. The maternal LOEL was based on the increased incidence of mortality. No developmental toxicity was present at the highest dose tested (1500 mg/kg/day).

	NOEL	LOEL
Maternal	500 mg/kg/day	1000 mg/kg/day
Developmental	1500 mg/kg/day	> 1500 mg/kg/day

CLASSIFICATION: Core - supplementary

This is not a guideline study.

I. Materials and Methods

A. Test compound: S-23031, technical Description: brown powder Batch #: PYG-88092-M Purity: 94.4% Contaminants: list in CBI appendix.

B. Dose Preparation: Test article was suspended in 0.5% methylcellulose (Lot No. 77F-0601, Sigma Chemical Co., St. Louis, MO) in water (Polar Distilled Water, Lot No. 0814912, All Pure Spring Water, Inc., Oakland, MD). Samples from the top, middle and bottom of all the dosing solutions were analyzed and found to be homogeneous (relative standard deviations 1.2 to 3.0%) and within 84.4 and 119% of the nominal concentration.

C. Test Animal: Species: Rabbit Strain: Female, Hra:(NZW)SPF Source: Hazleton Research Products, Inc., Denver, PA Age: 5.5 months Weight: 3177 to 4344 g.

D. Study Design: This study was designed to assess the developmental toxicity potential of S-23031 when administered by gavage to rabbits on gestation days 7 through 19, inclusive.

1. Mating: Animals were mated on a 1:1 basis and when copulation took place, the female was examined for traces of white fluid on the vulva. When the finding was negative, a second mating attempt with the same male was carried out. Following a successful mating, the female was injected with 250 I.U. of human chorionic gonadotropin. If, after 20 minutes, there was no confirmed mating, the female was mated with a second, randomly selected male.

2. Group Arrangement: The animals were randomly assigned to the following test groups:

Table 1: Animal Assignment to Study Groups

Test Group		Dosage (mg/kg/day)	Number Assigned
Control	(CON)	0	6
Low	(LDT)	300	6
Mid 1	(MDT1)	500	6
Mid 2	(MDT2)	1000	6
High	(HDT)	1500	6

3. Dosing: Concentrations of test article in the dosing solutions varied with dose (dose volume was kept constant at 5.0 ml/kg). The most recent body weights of the animals weighed on gestation days (gd) 0, 7, 9, 11, 15, 19, 20, 24 and 29 were used for determination

of the dose volume. Animals were dosed from gd 7 through gd 19, inclusive.

4. Observations

a. Maternal Observations and Evaluations: Animals were checked twice daily for signs of toxicity, mortality and moribundity. Does were sacrificed on gd 29 and examined for gross abnormalities of the thoracic, abdominal or pelvic viscera. Animals which died or were sacrificed moribund during the study were also examined.

b. Fetal Evaluations: Live fetuses were dissected from the uterus, weighed, and examined for external morphological abnormalities. Following the examination, each fetus was euthanized with an intraperitoneal injection of sodium pentobarbital, sexed, and saved in 10% neutral-buffered formalin.

E. Historical Control Data: Historical control data were not provided to allow comparison with concurrent controls.

F. Statistical Analysis: Data were analyzed for homogeneity of variances using Levene's Test. If found to be homogeneous, an analysis of variance (ANOVA) was performed. A rank transformation was performed on heterogeneous data and then reevaluated. If the transformed data were homogeneous, ANOVA was carried out. A significant ANOVA result was followed by Dunnett's Test to determine significant differences between the control and treated groups. Mean live fetal weights were further analyzed using one-way analysis of covariance, with the number of fetuses in each litter used as the covariant.

G. Compliance: Signed and dated GLP and Quality Assurance statements were provided.

H. Flagging Statement: The sponsor applied the criteria of 40 CFR 158.34 for flagging studies for potential adverse effects to the results of this study. This study neither meets nor exceeds any of the applicable criteria.

II. Results

A. Maternal Toxicity

1. Mortality: Treatment-related lethality was observed in the three highest dose groups. On day 29 one animal in the 500 mg/kg/day group died. Three animals in the 1000 mg/kg/day group died, one each on days 16, 21 and 24. At 1500 mg/kg/day, five animals died, one each on days 20, 21 and 22 and two on day 24.

2. Clinical Observations: Clinical observations noted during gestation included one thin animal in the 500 mg/kg/day group and one languid animal in the 1500 mg/kg/day group.

3. Body Weight: The only significant changes in body weight occurred on days 15 and 19 for animals in the highest dose group (Table 2).

Table 2: Mean Maternal Body Weights.

Days	CON	LDT	MDT1	MDT2	HDT
15	4157	4167	3770	4081	3563*
19	4087	4183	3706	4021	3344*

* $p \leq 0.05$

4. Food Consumption: Maternal food consumption was measured throughout the gestation period at two day intervals. The only significant change was noted on days 17 to 19 (Table 3).

Table 3: Food Consumption Data (g/animal/day)

Days	CON	LDT	MDT1	MDT2	HDT
17-19	142.2	161.8	85.5	115.1	22.8*

* $p \leq 0.05$

5. Gross Pathology: Necropsies were performed on gd 29 on all surviving maternal animals. The thoracic, abdominal and pelvic viscera were examined and the pregnancy status confirmed.

6. Cesarean Section Data: The cesarean section data are presented in Table 4, below. There were no statistically significant, treatment-related effects on any of the parameters measured.

B. Developmental Toxicity: The number of malformations and variations detected during external examinations of the fetuses is summarized in Table 5, below. No dose-related differences were noted in the incidence of malformation and variations observed in this study. The number of fetuses and litters with malformations and variations were comparable between the study groups.

Table 4: Cesarean Section Data

	CON	LDT	MDT1	MDT2	HDT
Total Assigned	6	6	6	6	6
No. (%) Gravid	5 (83)	5 (83)	5 (83)	4 (67)	6 (100)
With Resorptions Only	0	0	0	0	0
With Nonlive Fetuses Only	0	0	0	0	0
With Viable Fetuses	5	5	4	3	1
Maternal Wastage					
Total No. Died	0	0	1	3	5
No. Died Gravid	0	0	1	1	5
No. Died Nongravid	0	0	0	2	0
No. Nongravid	1	1	1	0	0
No. Aborted	0	0	0	0	0
No. Premature Delivery	0	0	0	0	0
Corpora Lutea/Doe	10.2	10.4	8.3	10.0	9.0
Implantation Sites/Litter	8.4	7.8	7.0	9.7	8.0
% Preimplantation Loss	15.0	24.9	18.6	3.7	11.1
No. (%) Resorptions/Litter - Total	2.4 (26.4)	0.2 (2.2)	0.3 (6.3)	3.7 (34.8)	0.0 (0.0)
- Early	1.0 (10.9)	0.0 (0.0)	0.3 (6.3)	1.0 (10.2)	0.0 (0.0)
- Late	1.4 (15.6)	0.2 (2.2)	0.0 (0.0)	2.7 (24.5)	0.0 (0.0)
No. (%) Live Fetuses/Litter - All	6.0 (73.6)	6.8 (88.9)	6.8 (93.8)	6.0 (65.2)	8.0 (100.0)
- Male	3.0 (42.2)	2.8 (41.1)	3.0 (35.3)	3.0 (62.4)	4.0 (50.0)
- Female	3.0 (42.2)	4.0 (58.9)	3.8 (64.7)	3.0 (37.6)	4.0 (50.0)
Sex Ratio (Male:Female)	50:50	41:59	44:56	50:50	50:50
Mean Fetal Body Weight/Litter - All	40.88	41.96	42.15	41.38	48.33
- Male	41.13	42.29	42.20	40.98	49.90
- Female	44.47	44.08	39.65	44.14	47.24

Table 5: External Fetal Examinations - Malformations and Variations

	CON	LDT	MDT1	MDT2	HDT
No. Live Fetuses Examined	30	34	27	18	8
No. of Litters Examined	5	5	4	3	1
External Malformations					
No. (%) of Malformed Fetuses	0 (0.0)	4 (11)	0 (0.0)	0 (0.0)	0 (0.0)
No. (%) of Litte s with Malformations	0 (0.0)	2 (40)	0 (0.0)	0 (0.0)	0 (0.0)
External Variations					
No. (%) of Fetuses with Variations	0 (0.0)	2 (5.3)	0 (0.0)	0 (0.0)	0 (0.0)
No. (%) of Litters with Variations	0 (0.0)	1 (20)	0 (0.0)	0 (0.0)	0 (0.0)

III. Discussion/Conclusions

A. Maternal Toxicity: Maternal toxicity was expressed as increased incidence of mortality in the three highest dose groups. There was a dose dependent increase in maternal mortality. One, three and five maternal deaths were observed in the 500, 1000 and 1500 mg/kg/day dose groups, respectively.

Cesarean section data was comparable between all of the study groups. During gestation days 15 and 19, there was a significant, decrease in the mean maternal body weight in the 1500 mg/kg/day group; this loss in body weight correlated with significant decreases in food consumption during the same time period.

B. Developmental Toxicity: No significant differences in the incidence of external malformations and variations were detected. External malformations and variations were present only in the 300 mg/kg/day group and was limited to a single litter. The observed external morphological abnormalities were not treatment-related.

C. Conclusions: The developmental effects of S-23031 on rabbits was evaluated in a range-finding study in which the animals were dosed at 0, 300, 500, 1000 or 1500 mg/kg/day throughout the organogenesis period. The maternal LOEL was based on the increased incidence of mortality. No developmental toxicity was present at the highest dose tested (1500 mg/kg/day).

	NOEL	LOEL
Maternal	500 mg/kg/day	1000 mg/kg/day
Developmental	1500 mg/kg/day	> 1500 mg/kg/day

Classification: Core - supplementary

This is not a guideline study.

Reviewed by: Robert F. Fricke, Ph.D. Robert F. Fricke 11 May 92
Section IV, Tox. Branch II (H7509C)
Secondary Reviewer: Elizabeth A. Doyle, Ph.D. E.A. Doyle 5/1/92
Section IV, Tox. Branch II (H7509C)

DATA EVALUATION RECORD 009587

STUDY TYPE: Teratology - Developmental Toxicity - Rabbit (83-3)

P.C. CODE: 128724

MRID NO.: 421698-30

TEST MATERIAL: S-23031

SYNONYMS: Pentyl 2-chloro-4-fluoro-5-[(3,4,5,6-tetrahydro) phthalimido] phenoxyacetate

STUDY NUMBER: HLA 343-2_

SPONSOR: Sumitomo Chemical Co., Ltd
Kitahama, 4-Chome 5-33, Chuo-Ku
Osaka, Japan

TESTING FACILITY: Hazleton Laboratories America, Inc.
9200 Leesburg Turnpike
Vienna, VA 22182

TITLE OF REPORT: Rabbit Teratology Study with S-23031

AUTHOR: J.K. Lemen

REPORT ISSUED: 22 February 1991

CONCLUSIONS: The developmental effect of S-23031, at dosages of 0, 100, 200, 400 or 800 mg/kg/day, was evaluated in rabbits throughout the organogenesis period. The maternal LOEL was based on the increased incidence of mortality at 800 mg/kg/day. No developmental toxicity was present at the highest dose tested (800 mg/kg/day).

	NOEL	LOEL
Maternal	400 mg/kg/day	800 mg/kg/day
Developmental	800 mg/kg/day	> 800 mg/kg/day

CLASSIFICATION: Core - Guideline

This study does satisfy guideline requirements (83-3) for Teratology - Developmental Toxicity in the rabbit.

I. Materials and Methods

A. Test compound: S-23031, technical Description: brown powder Batch #: PYG-88092-M Purity: 94.4% Contaminants: list in CBI appendix.

B. Dose Preparation: Test article was suspended in 0.5% methylcellulose (Lot No. 128F-0451, Sigma Chemical Co., St. Louis, MO) in water (Polar Distilled Water, Lot No. 0617911, All Pure Spring Water, Inc., Oakland, MD). Samples from the top, middle and bottom of all the dosing solutions were analyzed and found to be homogeneous (relative standard deviations of 0.68 to 1.3%) and within 92.6 and 104% of the nominal concentration.

C. Test Animal: Species: Rabbit Strain: Female, Hra:(NZW)SPF Source: Hazleton Research Products, Inc., Denver, PA Age: 5.25 months Weight: 2834 to 4432 g.

D. Study Design: This study was designed to assess the developmental toxicity potential of S-23031 when administered by gavage to rabbits on gestation days 7 through 19, inclusive.

1. Mating: One female was placed in a cage with a single male. Upon observation of the copulatory act, the female was removed from the cage and the vulva checked for traces of white fluid. When the finding was negative, the female was returned to the same male for a second mating attempt. Once the copulatory act was observed and confirmed, the female was injected with 250 I.U. of human chorionic gonadotropin. When mating was not confirmed after 20 minutes, the female was introduced to a second, randomly selected male.

2. Group Arrangement: The animals were randomly assigned to the following test groups:

Table 1: Animal Assignment to Study Groups

Test Group		Dosage (mg/kg/day)	Number Assigned
Control	(CON)	0	17
Low	(LDT)	100	17
Middle 1	(MDT1)	200	17
Middle 2	(MDT2)	400	17
High	(HDT)	800	17

3. Dosing: Concentrations of test article in the dosing solutions varied with dose (dose volume was kept constant at 5.0 ml/kg). The most recent animal body weight, determined on gestation days (gd) 0, 7, 9, 11, 15, 19, 20, 24 and 29, was used for determination of

the dose volume. Animals were dosed from gd 7 through gd 19, inclusive.

4. Observations

a. Maternal Observations and Evaluations: Animals were checked twice daily for signs of toxicity, mortality and moribundity. Does were sacrificed on gd 29 and examined for gross abnormalities of the thoracic, abdominal and pelvic viscera. Animals which died or were sacrificed moribund during the study were also examined.

b. Fetal Evaluations: Live fetuses were dissected from the uterus, weighed and examined for morphological abnormalities. Following external examination, each fetus was euthanized with an intraperitoneal injection of sodium pentobarbital, sexed, and examined for visceral and skeletal abnormalities.

E. Historical Control Data: Historical control data were not provided to allow comparison with concurrent controls.

F. Statistical Analysis: Data were analyzed for homogeneity of variances using Levene's Test. If found to be homogeneous, an analysis of variance (ANOVA) was performed. Heterogeneous data were reevaluated after a rank transformation was performed and if homogeneous an ANOVA was carried out. A significant ANOVA result was followed by Dunnett's Test to determine significant differences between the control and treatment groups. Mean live fetal weights were further analyzed using one-way analysis of covariance, with the number of fetuses in each litter used as the covariant. Incidence data were analyzed using the Cochran-Armitage test for linear trend and the Fisher-Irwin exact test.

G. Compliance: Signed and dated GLP and Quality Assurance statements were provided.

H. Flagging Statement: The sponsor applied the criteria of 40 CFR 158.34 for flagging studies for potential adverse effects to the results of this study. This study neither meets nor exceeds any of the applicable criteria.

II. Results

A. Maternal Toxicity

1. Mortality: Four animals in the 800 mg/kg/day group were found dead, one each on days 13 and 17, and two on day 26. One of the animals that died on day 26 appeared thin on day 24 and aborted prior to death.

Two animals in the control group died, one due to gavage error on day 16 and the other on day 26.

2. Clinical Observations: The observed clinical signs did not occur in a dose-related pattern.

3. Body Weight: Comparison of the control and treatment groups showed that there were no significant differences in mean maternal body weight, body weight gain, gravid uterine weight, and corrected maternal weight.

4. Food Consumption: There were no significant treatment-related changes in maternal food consumption.

5. Gross Pathology: Necropsies were performed on gd 29 on all surviving maternal animals. The thoracic, abdominal and pelvic viscera were examined and the pregnancy status confirmed. There were no significant treatment-related pathological findings. Incidental changes found at the scheduled sacrifice, included dark-red glandular and nonglandular mucosae of the stomach in one animal in the 400 mg/kg/day group and two animals in the 800 mg/kg/day group; a single animal in the 800 mg/kg/day group had reddening of the glandular mucosa.

6. Cesarean Section Data: The cesarean section data are presented in Table 2, below. There were no statistically significant treatment-related effects on any of the parameters evaluated.

B. Developmental Toxicity: The incidence of external, visceral and skeletal malformations and variations is summarized in Tables 3 and 4. No statistically significant changes in the incidence of malformations were found. The malformations present were of low frequency and comparable between the study groups. While no external variations were noted in any of the groups, significant changes in the incidence of visceral and skeletal variations were found. Significant visceral variations noted were increased litter incidence of accessory subclavian arising anterior or posterior to the left subclavian in the 400 mg/kg/day group. The 800 mg/kg/day group did not show a statistically significant increase, however, a significant, positive trend was noted with increasing dose of test article. Other visceral variations included significant decrease in the incidence of a small and/or missing intermediate lobe of the lung in the 400 mg/kg/day group. Significant skeletal variations included a decreased incidence of 26 presacral vertebrae in the 100, 400 and 800 mg/kg/day groups and increased incidence of incomplete or unossified hyoid wings and unossified 5th sternebrae in the 400 mg/kg/day group.

Table 2: Cesarean Section Data

	CON	LDT	MDT1	MDT2	HDT
Total Assigned	17	17	17	17	17
No. (%) Gravid	16 (94)	12 (71)	15 (88)	17 (100)	15 (88)
With Nonviable Fetuses Only	2	0	0	1	2
With Viable Fetuses	12	11	14	16	9
Maternal Wastage					
Total No. Died	3	0	0	0	4
Died Gravid	1	0	0	0	2
Died Nongravid	1	0	0	0	1
Accidental Death	1	0	0	0	0
Aborted/Died	0	0	0	0	1
No. Nongravid	1	5	2	0	1
No. Aborted	0	1	0	0	1
No. Premature Delivery	0	0	1	0	0
Corpora Lutea/Doe	10.2	10.8	11.1	10.8	9.1
Implantation Sites/Litter	7.7	9.1	7.9	7.9	6.4
% Preimplantation Loss	25.2	15.1	25.2	26.4	3?.3
No. (%) Resorptions/Litter - Total	1.3 (27.2)	1.2 (12.6)	0.9 (11.0)	1.1 (17.6)	0.4 (19.1)
- Early	1.2 (26.5)	0.7 (8.4)	0.7 (10.0)	0.9 (15.7)	0.4 (19.1)
- Late	0.1 (0.7)	0.5 (4.2)	0.1 (1.0)	0.2 (1.9)	0.0 (0.0)
No. (%) Live Fetuses/Litter - All	6.4 (72.8)	7.9 (87.4)	7.1 (89.0)	6.8 (82.4)	6.0 (80.9)
- ♂	3.7 (48.1)	3.6 (46.6)	3.6 (48.8)	4.1 (56.6)	3.6 (48.1)
- ♀	3.8 (51.9)	4.3 (53.4)	3.5 (51.2)	3.1 (43.4)	3.8 (51.9)
Sex Ratio (Male:Female)	49:51	46:54	51:49	57:43	48:52
Mean Fetal Body Weight (g)/Litter - All	43.47	42.63	43.56	45.22	45.04
- ♂	45.95	41.79	44.20	45.79	46.11
- ♀	41.13	43.36	42.65	44.62	44.31

Table 3: Fetal Examinations - Malformations

	CON	LDT	MDT1	MDT2	HDT
No. Fetuses Examined	90	87	95	115	66
No. Litters Examined	12	11	14	16	9
External Malformations					
Fetal Incidence, No (%)	3 (3.3)	1 (1.1)	1 (1.1)	1 (0.9)	0 (0.0)
Litter Incidence, No. (%)	3 (25)	1 (9.1)	1 (7.1)	1 (6.3)	0 (0.0)
Visceral Malformations					
Fetal Incidence, No (%)	3 (3.3)	1 (1.1)	3 (3.2)	2 (1.7)	3 (4.5)
Litter Incidence, No. (%)	2 (17)	1 (9.1)	3 (21)	2 (13)	1 (11)
Skeletal Malformations					
Fetal Incidence, No. (%)	4 (4.4)	8 (9.2)	3 (3.2)	4 (3.5)	1 (1.5)
Litter Incidence, No. (%)	4 (33)	5 (45)	3 (21)	3 (19)	1 (11)

Table 4: Fetal Examinations - Variations

CON	LDT	MDT1	MDT2	HDT	
No. Fetuses Examined	90	87	95	115	66
No. Litters Examined	12	11	14	16	9
External Variations					
Fetal Incidence, No. (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Litter Incidence, No. (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Visceral Variations					
Fetal Incidence, No. (%)	13 (14)	11 (13)	16 (17)	21 (18)	14 (21)
Intermediate Lobe of Lung Small/Missing	6 (6.7)	2 (2.3)	4 (4.2)	1* (0.9)	1 (1.5)
Litter Incidence, No. (%)	7 (58)	5 (45)	10 (71)	12 (75)	7 (78)
Accessory Subclavian Anterior/ Posterior to Left Subclavian	2 (17)	4 (36)	4 (29)	9* (56)	5 (56)
Skeletal Variations					
Fetal Incidence, No. (%)	76 (84)	74 (85)	80 (84)	92 (80)	51 (77)
26 Presacral Vertebrae	34 (38)	19* (22)	30 (32)	20** (17)	14* (21)
5th Sternebrae Unossified	2 (2.2)	4 (4.6)	4 (4.2)	11* (9.6)	2 (3.0)
Incomplete/Unossified Hyoid Wing(s)	0 (0.0)	4 (4.6)	1 (1.1)	7* (6.1)	0 (0.0)
Litter Incidence, No. (%)	12 (100)	11 (100)	14 (100)	16 (100)	9 (100)

* $p \leq 0.05$, ** $p \leq 0.01$

III. Discussion/Conclusions

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A. Maternal Toxicity: Maternal toxicity was expressed as increased incidence of mortality in the 800 mg/kg/day group. There were no significant, treatment-related changes in maternal body weight, body weight gain, gravid uterine weight or corrected maternal body weight. Food consumption was comparable between all dose groups. No significant, treatment-related changes were noted in the cesarean section data; each parameter measured was comparable in all the study groups.

B. Developmental Toxicity: The only significant finding observed was an increased litter incidence of the accessory subclavian arising anterior or posterior to the left subclavian in the 400 mg/kg/day group. Although the 800 mg/kg/day group was not significantly different from the control, there was a significant positive trend with increasing dose. This variation was not considered to adversely affect blood flow or survival. With the absence of any significant differences between control and treatment groups in the incidence of any malformations, the test compound is not developmentally toxic.

C. Conclusions: The developmental effect of S-23031, at dosages of 0, 100, 200, 400 or 800 mg/kg/day, was evaluated in rabbits throughout the organogenesis period. The maternal LOEL was based on the increased incidence of mortality at 800 mg/kg/day. No developmental toxicity was present at the highest dose tested (800 mg/kg/day).

	NOEL	LOEL
Maternal	400 mg/kg/day	800 mg/kg/day
Developmental	800 mg/kg/day	> 800 mg/kg/day

Classification: core - Guideline

This study does satisfy guideline requirements (83-3) for Teratology - Developmental Toxicity in the rabbit.

005587

Reviewed by: Robert F. Fricke, Ph.D. Robert F. Fricke 30 Apr 92
Section IV, Tox. Branch II (H7509C)
Secondary Reviewer: Elizabeth A. Doyle, Ph.D. E.A. Doyle 5/1/92
Section IV, Tox. Branch II (H7509C)

DATA EVALUATION RECORD

STUDY TYPE: Range Finder - Teratology - Developmental Toxicity - Rat

P.C. CODE: 128724

MRID NO.: 421698-31

TEST MATERIAL: S-23031

SYNONYMS: Pentyl 2-chloro-4-fluoro-5-[(3,4,5,6-tetrahydro) phthalimido] phenoxyacetate

STUDY NUMBER: HLA 343-222

SPONSOR: Sumitomo Chemical Co., Ltd
Kitahama, 4-Chome 5-33, Chuo-Ku
Osaka, Japan

TESTING FACILITY: Hazleton Laboratories America, Inc.
9200 Leesburg Turnpike
Vienna, VA 22182

TITLE OF REPORT: Dose-Finding Study for Teratology in Rats with S-23031

AUTHOR: S.L. Morseth

REPORT ISSUED: 22 May 1990

CONCLUSIONS: The developmental effects of S-23031 on rats was evaluated in a range-finding study. Animals were dosed at 0, 300, 500, 1000 or 1500 mg/kg/day throughout the organogenesis period. No maternal or developmental toxicity was present at the highest dose tested (1500 mg/kg/day).

	NOEL	LOEL
Maternal	1500 mg/kg/day	> 1500 mg/kg/day
Developmental	1500 mg/kg/day	> 1500 mg/kg/day

CLASSIFICATION: Core - Supplementary

This is not a guideline study.

I. Materials and Methods

A. Test compound: S-23031, technical Description: brown powder Batch #: PYG-88092-M Purity: 94.4% Contaminants: list in CBI appendix.

B. Dose Preparation: Test article was suspended in 0.5% methylcellulose (Lot No. 77F-0601, Sigma Chemical Co., St. Louis, MO) in water (Polar Distilled Water, Lot No. 229902, All Pure Spring Water, Inc. Oakland, MD). Samples were within 94.9 and 113% of the nominal concentration.

C. Test Animal: Species: Rat, Strain: Female, Crl:CD BR Source: Charles River Laboratories, Inc., Raleigh, NC Age (weeks): 10 - 12 (original phase), 11 - 12 (repeat phase) Weight (g): 232 - 295 (original phase), 250 - 272 (repeat phase).

D. Study Design: This study was designed to assess the developmental toxicity potential of S-23031 when administered by gavage to rats on gestation days 6 through 15, inclusive.

1. Mating: One female was placed in a cage with a single male until mating was confirmed. Daily examinations were performed to detect the presence of sperm (vaginal smear) or copulatory plug. The day of observation of sperm or copulatory plug was designated as gestation day (gd) 0.

2. Group Arrangement: The animals were randomly assigned to the following test groups:

Table 1: Animal Assignment to Study Groups

Study Group[a]	Dosage (mg/kg/day)	Number Assigned
Control (CON-O, CON-R)	0	6,6
Low (LDT)	300	6
Mid 1 (MDT1)	500	6
Mid 2 (MDT2)	1000	6
High (HDT-O, HDT-R)	1500	6,6

[a] Because the high dose may not have been properly resuspended each day, a portion of study was repeated to include another control group (CON-R) and another high dose group (HDT-R). The original control and high dose groups are designated as CON-O and HDT-O, respectively.

3. Dosing: Concentrations of test article in the dosing solutions varied with dose (dose volume was kept

constant at 5.0 ml/kg). The most recent body weights of the animals weighed on gd 0, 6, 8, 10, 12, 15, 16 and 20 were used for determination of the dose volume. Animals were dosed from gd 6 through gd 15, inclusive.

4. Observations

a. Maternal Observations and Evaluations: Animals were checked twice daily for signs of toxicity, mortality and moribundity. Animals were sacrificed on gd 20 and examined for gross abnormalities of the thoracic, abdominal and pelvic viscera. Animals which died or were sacrificed moribund during the study were also examined.

b. Fetal Evaluations: Live fetuses were dissected from the uterus, weighed and examined for external morphological abnormalities. Following the examination, each fetus was euthanized with an intraperitoneal injection of sodium pentobarbital, sexed, and preserved in formalin.

E. Historical Control Data: Historical control data were not provided to allow comparison with concurrent controls.

F. Statistical Analysis: Data were analyzed for homogeneity of variances using Levene's Test. If found to be homogeneous, an analysis of variance (ANOVA) was performed. Heterogeneous data first underwent a rank transformation, followed by an ANOVA if homogeneous. A significant ANOVA result was followed by Dunnett's Test to determine significant differences between the control and treated groups. Mean live fetal weights were further analyzed using one-way analysis of covariance, with the number of fetuses in each litter used as the covariant.

G. Compliance: Signed and dated GLP and Quality Assurance statements were provided.

H. Flagging Statement: The sponsor applied the criteria of 40 CFR 158.34 for flagging studies for potential adverse effects to the results of this study. This study neither meets nor exceeds any of the applicable criteria.

II. Results

A. Maternal Toxicity

1. Mortality: All animals in both the original and repeat phases survived until the scheduled sacrifice.

2. Clinical Observations: No treatment-related clinical observations were noted during gestation for

either the original or repeat phases of the study.

3. Body Weight: No significant changes between the control and treated groups were noted in either the mean body weight or the mean body weight gain.

4. Food Consumption: No statistically significant differences in food consumption were found between the control and treatment groups.

5. Gross Pathology: Necropsies were performed on gd 20 on all surviving maternal animals. The thoracic, abdominal and pelvic viscera were examined and the pregnancy status confirmed. There were no significant treatment-related findings.

6. Cesarean Section Data: The cesarean section data are presented in Tables 2 and 3 for the original and repeat phases of the study, respectively. There were no statistically significant treatment-related effects on any of the parameters measured.

B. Developmental Toxicity: The incidence of malformations and variations detected during external examinations of the fetuses is summarized in Table 4, below. No biologically meaningful differences were noted. The number of fetuses and litters with malformations and variations were comparable between all the study groups.

III. Discussion/Conclusions

A. Maternal Toxicity: No maternal toxicity was present at the highest dose tested (1500 mg/kg/day).

B. Developmental Toxicity: No significant differences in the incidence of external malformations were detected in any of the study groups.

C. Conclusions: The developmental effects of S-23031 on rats was evaluated in a range-finding study. Animals were dosed at 0, 300, 500, 1000 or 1500 mg/kg/day throughout the organogenesis period. No maternal or developmental toxicity was present at the highest dose tested (1500 mg/kg/day).

	NOEL	LOEL
Maternal	1500 mg/kg/day	> 1500 mg/kg/day
Developmental	1500 mg/kg/day	> 1500 mg/kg/day

Classification: Core - Supplementary

This is not a guideline study.

Table 2: Cesarean Section Data - Original Phase of Study

	CON-O	LDT	MDT1	MDT2	HDT-O
Total Assigned	6	6	6	6	6
No. (%) Gravid	6 (100)	6 (100)	6 (100)	5 (83)	6 (100)
With Resorptions Only	0	0	0	0	0
With Nonlive Fetuses Only	0	0	0	0	0
With Viable Fetuses	6	6	6	5	6
Maternal Wastage					
No. Died Gravid	0	0	0	0	0
No. Died Nongravid	0	0	0	0	0
No. Nongravid	0	0	0	1	0
No. Aborted	0	0	0	0	0
No. Premature Delivery	0	0	0	0	0
Mean Corpora Lutea/Doe	16.7	18.3	16.2	17.2	17.7
Mean Implantation Sites/Litter	15.0	17.2	15.3	15.4	16.7
% Preimplantation Loss	8.6	6.2	4.7	9.9	5.9
Mean (%) Resorptions/Litter - Total	0.5 (3.7)	1.2 (7.0)	0.2 (1.0)	1.0 (6.8)	1.5 (8.3)
- Early	0.3 (2.6)	1.2 (7.0)	0.2 (1.0)	0.8 (5.0)	1.5 (8.3)
- Late	0.2 (1.1)	0.0 (0.0)	0.0 (0.0)	0.2 (1.8)	0.0 (0.0)
Mean (%) Live Fetuses/Litter - All	14.5 (96)	16.0 (93)	15.2 (99)	14.4 (93)	15.2 (92)
- Male	7.0 (48)	8.3 (51)	7.2 (47)	7.4 (53)	9.0 (60)
- Female	7.5 (52)	7.7 (49)	8.0 (53)	7.0 (47)	6.2 (40)
Sex Ratio (Male:Female)	48:52	52:48	47:53	51:49	59:41
Mean Fetal Body Weight(g)/litter - All	3.57	3.48	3.75	3.79	3.87
- Male	3.64	3.57	3.84	3.91	3.97
- Female	3.50	3.39	3.67	3.63	3.71

Table 3: Cesarean Section Data - Repeat Phase of Study

	CON-R	HDT-R
Total Assigned	6	6
No. (%) Gravid	6 (100)	6 (100)
With Resorptions Only	0	0
With Nonlive Fetuses Only	0	0
With Viable Fetuses	6	6
Maternal Wastage		
No. Died Gravid	0	0
No. Died Nongravid	0	0
No. Nongravid	0	0
No. Aborted	0	0
No. Premature Delivery	0	0
Corpora Lutea/Doe	18.5	17.5
Implantation Sites/Litter	16.7	16.2
% Preimplantation Loss	10.0	7.4
Mean (%) Resorptions/Litter - Total	0.5 (3.0)	0.5 (3.1)
- Early	0.5 (3.0)	0.5 (3.1)
- Late	0.0 (0.0)	0.0 (0.0)
Mean (%) Live Fetuses/Litter - All	16.2 (97)	15.7 (97)
- Male	8.3 (53)	7.3 (47)
- Female	7.8 (47)	8.3 (53)
Sex Ratio (Male:Female)	52:48	47:53
Mean Fetal Body Weight(g)/litter - All	3.53	3.55
- Male	3.66	3.68
- Female	3.39	3.43

Table 4: External Fetal Examinations - Malformations and Variations

	CON	LDT	MDT1	MDT2	HDT
Original Phase					
No. Live Fetuses Examined	87	96	91	72	91
No. of Litters Examined	6	6	6	5	6
External Malformations					
No. (%) of Malformed Fetuses	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
No. (%) of Litters with Malformations	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Repeat Phase					
No. Live Fetuses Examined	97	--	--	--	94
No. of Litters Examined	6	--	--	--	6
External Malformations					
No. (%) of Malformed Fetuses	0 (0.0)	--	--	--	0 (0.0)
No. (%) of Litters with Malformations	0 (0.0)	--	--	--	0 (0.0)

Reviewed by: Robert F. Fricke, Ph.D. Robert F. Fricke 1 May 92
Section IV, Tox. Branch II (H7509C)
Secondary Reviewer: Elizabeth A. Doyle, Ph.D. E.A. Doyle 5/5/92
Section IV, Tox. Branch II (H7509C)

DATA EVALUATION RECORD

STUDY TYPE: Teratology - Developmental Toxicity - Rat (83-3)

009587

P.C. CODE: 128724

MRID NO.: 421698-32

TEST MATERIAL: S-23031

SYNONYMS: Pentyl 2-chloro-4-fluoro-5-[(3,4,5,6-tetrahydro) phthalimido] phenoxyacetate

STUDY NUMBER: HLA 343-223

SPONSOR: Sumitomo Chemical Co., Ltd
Kitahama, 4-Chome 5-33, Chuo-Ku
Osaka, Japan

TESTING FACILITY: Hazleton Laboratories America, Inc.
9200 Leesburg Turnpike
Vienna, VA 22182

TITLE OF REPORT: Rat Teratology Study with S-23031

AUTHOR: J.K. Lemen

REPORT ISSUED: 22 February 1991

CONCLUSIONS: The developmental effects of S-23031 were evaluated in rats dosed at 0, 50, 500 or 1500 mg/kg/day throughout the organogenesis period. No maternal or developmental toxicity was present at the highest dose tested (1500 mg/kg/day).

	NOEL	LOEL
Maternal	1500 mg/kg/day	> 1500 mg/kg/day
Developmental	1500 mg/kg/day	> 1500 mg/kg/day

CLASSIFICATION: Core - Guideline

This study does satisfy guideline requirements (83-3) for Teratology - Developmental Toxicity in the rat.

I. Materials and Methods

A. Test compound: S-23031, technical Description: brown powder Batch #: PYG-88092-M Purity: 94.4% Contaminants: list in CBI appendix.

B. Dose Preparation: Test article was suspended in an aqueous (Polar Distilled Water, Lot No. 061791, All Pure Spring Water, Inc., Oakland, MD) solution of 0.5% methylcellulose (Lot No. 128F 0451, Sigma Chemical Co., St. Louis, MO). Samples from the top, middle and bottom of all the dosing solutions were analyzed and found to be homogeneous (relative standard deviations 0.11 and 1.6%) and within 89.9 and 101% of the nominal concentration.

C. Test Animal: Species: Rat Strain: Female, Crl:CD BR Source: Charles River Laboratories, Inc., Raleigh, NC Age (weeks): 10-12 Weight (g): 206-293.

D. Study Design: This study was designed to assess the developmental toxicity potential of S-23031 when administered by gavage to rats on gestation days 6 through 15, inclusive.

1. Mating: One female was placed in a cage with a single male until mating was confirmed by the presence of sperm (vaginal smear) or copulatory plug. The day of observation of sperm or copulatory plug was designated as gestation day (gd) 0.

2. Group Arrangement: The animals were randomly assigned to the following test groups:

Table 1: Animal Assignment to Study Groups

Test Group		Dosage (mg/kg/day)	Number Assigned
Control	(CON)	0	25
Low	(LDT)	50	25
Mid	(MDT)	500	25
High	(HDT)	1500	25

3. Dosing: Concentrations of test article in the dosing solutions varied with dose (dose volume was kept constant at 5.0 ml/kg). The most recent body weights of the animals weighed on gd 0, 6, 8, 10, 12, 15, 16 and 20 were used for determination of the dose volume. Animals were dosed from gd 6 through gd 15, inclusive.

4. Observations

a. Maternal Observations and Evaluations: Animals were checked twice daily for signs of toxicity, mortality and moribundity. Animals were sacrificed on gd 20 and examined for gross abnormalities of the thoracic, abdominal and pelvic viscera. Animals which died or were sacrificed moribund during the study were also examined.

b. Fetal Evaluations: Live fetuses were dissected from the uterus, weighed and examined for external, skeletal and visceral morphological abnormalities. All of the fetuses were examined for external abnormalities. Two groups of fetuses per litter per dose group were randomly selected; approximately one-half of the fetuses was subjected to visceral examination; the remaining fetuses were eviscerated, cleared and stained with Alizarin Red S, and examined for skeletal malformations and variations. All fetuses were retained in either Bouin's fixative or glycerin with thymol added as a preservative.

E. Historical Control Data: Historical control data were not provided to allow comparison with concurrent controls.

F. Statistical Analysis: Data were analyzed for homogeneity of variances using Levene's Test. If found to be homogeneous, an analysis of variance (ANOVA) was performed. Heterogeneous data underwent a rank transformation. If the transformed data were homogeneous, ANOVA was carried out. A significant ANOVA result was followed by Dunnett's Test to determine significant differences between the control and treated groups. Mean live fetal weights were further analyzed using one-way analysis of covariance, with the number of fetuses in each litter used as the covariant.

G. Compliance: Signed and dated GLP and Quality Assurance statements were provided.

H. Flagging Statement: The sponsor applied the criteria of 40 CFR 158.34 for flagging studies for potential adverse effects to the results of this study. This study neither meets nor exceeds any of the applicable criteria.

II. Results

A. Maternal Toxicity

1. Mortality: All animals survived until the scheduled sacrifice.

2. Clinical Observations: No treatment-related clinical observations were noted during gestation.

3. Body Weight: No significant changes were noted in either the mean body weight or the mean body weight gain between the control and treated groups. Gravid uterine weight and corrected maternal body weight were also similar in all groups.

4. Food Consumption: No statistically significant differences between the control and treated groups were found in food consumption.

5. Gross Pathology: Necropsies were performed on gd 20 on all surviving maternal animals. The thoracic, abdominal and pelvic viscera were examined and the pregnancy status confirmed. There were no significant treatment-related findings.

6. Cesarean Section Data: The cesarean section data are presented in Table 2, below. There were no statistically significant treatment-related effects on any of the parameters measured.

B. Developmental Toxicity: The numbers of fetal malformations and variations are summarized in Tables 3. No statistically significant differences were noted in the incidence of malformation and variation. The number of fetuses and litters with malformations and variations were comparable between the study groups.

III. Discussion/Conclusions

A. Maternal Toxicity: There was no significant dose-related maternal toxicity in any of the study groups. All animals survived until the scheduled sacrifice with no treatment-related clinical observations noted. No significant changes were noted in either the mean body weight, the mean body weight gain, gravid uterine weight, or corrected maternal weight between the control and treated groups. Food consumption was comparable between all of the groups. Cesarean section data did not show any treatment-related effects.

B. Developmental Toxicity: No significant differences in the incidence of external, visceral or skeletal abnormalities were detected between control and treated rats.

Table 2: Cesarean Section Data

	CON	LDT	MDT	HDT
Total Assigned	25	25	25	25
No. (%) Gravid	24 (96)	23 (92)	24 (96)	24 (96)
With Nonviable Fetuses Only	0	0	0	0
With Viable Fetuses	24	23	24	24
Maternal Wastage				
No. Died	0	0	0	0
No. Died Nongravid	0	0	0	0
No. Nongravid	1	2	1	1
No. Aborted	0	0	0	0
No. Aborted/Died	0	0	0	0
No. Premature Delivery	0	0	0	0
Corpora Lutea	18.5	18.2	18.0	18.0
Implantation Sites/Litter	16.3	16.0	15.9	16.2
% Preimplantation Loss	10.9	12.9	12.0	9.7
No. (%) Resorptions/Litter - Total	1.0 (6.2)	0.9 (7.1)	0.9 (6.8)	0.8 (5.1)
- Early	1.0 (6.2)	0.8 (6.6)	0.9 (6.8)	0.8 (5.1)
- Late	0.0 (0.0)	0.1 (0.5)	0.0 (0.0)	0.0 (0.0)
No. (%) Live Fetuses/Litter - All	15.3	15.1	15.0	15.4
- Male	7.7	7.8	7.5	7.8
- Female	7.6	7.3	7.5	7.5
Sex Ratio (Male:Female)	50:50	52:48	50:50	51:49
Mean Fetal Body Weight (g)/Litter - All	3.54	3.70	3.60	3.65
- Male	3.62	3.79	3.71	3.76
- Female	3.45	3.48	3.51	3.55

Table 3: Fetal Examinations - Malformations and Variations

	CON	LDT	MDT	HDT
External Abnormalities				
No. Fetuses Examined	368	347	359	369
No. Litters Examined	24	23	24	24
Malformations				
Fetal Incidence, No. (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Litter Incidence, No. (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Variations				
Fetal Incidence, No. (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Litter Incidence, No. (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Visceral Abnormalities				
No. Fetuses Examined	184	173	180	185
No. Litters Examined	24	23	24	24
Malformations				
Fetal Incidence, No. (%)	0 (0.0)	1 (0.6)	0 (0.0)	0 (0.0)
Litter Incidence, No. (%)	0 (0.0)	1 (4.3)	0 (0.0)	0 (0.0)
Variations				
Fetal Incidence, No. (%)	14 (7.6)	9 (5.2)	4 (2.2)	11 (5.9)
Litter Incidence, No. (%)	9 (38)	6 (26)	4 (17)	8 (33)
Skeletal Abnormalities				
No. Fetuses Examined	184	174	179	184
No. Litters Examined	24	22	24	24
Malformations				
Fetal Incidence, No. (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Litter Incidence, No. (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Variations				
Fetal Incidence, No. (%)	131 (71)	136 (78)	123 (69)	119 (65)
Litter Incidence, No. (%)	24 (100)	22 (100)	24 (100)	23 (96)

C. Conclusions: The developmental effects of S-23031 were evaluated in rats dosed at 0, 50, 500 or 1500 mg/kg/day throughout the organogenesis period. No maternal or developmental toxicity was present at the highest dose tested (1500 mg/kg/day).

	NOEL	LOEL
Maternal	1500 mg/kg/day	> 1500 mg/kg/day
Developmental	1500 mg/kg/day	> 1500 mg/kg/day

CLASSIFICATION: Core - Guideline

This study does satisfy guideline requirements (83-3) for Teratology - Developmental Toxicity in the rat.

Reviewed by: Robert F. Fricke, Ph.D. Robert F. Fricke 7 May 92
Section IV, Tox. Branch II (H7509C)
Secondary Reviewer: Elizabeth A. Doyle, Ph.D. E.A. Doyle 5/8/92
Section IV, Tox. Branch II (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: Range Finding - Two Generation Reproduction - Rat

009587

P.C. CODE: 128724

MRID NO.: 421698-34

TEST MATERIAL: S-23031

SYNONYMS: Pentyl 2-chloro-4-fluoro-5-[(3,4,5,6-tetrahydro) phthalimido] phenoxyacetate

STUDY NUMBER: WIL-118007

SPONSOR: Sumitomo Chemical Co., Ltd
Kitahama, 4-Chome 5-33, Chuo-Ku
Osaka 541 Japan

TESTING FACILITY: Wil Research Laboratories, Inc.
Ashland, OH 44805-9281

TITLE OF REPORT: A Dietary Dose Range-Finding Reproductive Toxicity Study of S-23031 in Rats

AUTHOR: M.D. Nemec

REPORT ISSUED: 6 June 1990

CONCLUSIONS: This range-finding study was designed to evaluate the reproductive and systemic toxicity of S-23031 administered to male and female rats for one generation. Dosages of 0, 1000, 5000, 10000 or 20000 ppm (equivalents to: 61, 302, 61/ and 1300 mg/kg/day for males and 72, 344, 707 and 1450 mg/kg/day females, prior to breeding; 76, 366, 765 and 1505 mg/kg/day during gestation; 153, 753, 1610 and 3055 mg/kg/day during lactation) did not elicit significant systemic or reproductive toxicity.

	NOEL	LOEL
Systemic	20000 ppm	> 20000 ppm
Reproductive	20000 ppm	> 20000 ppm

CLASSIFICATION: core - Supplementary

This is not a guideline study.

I. MATERIALS:

A. Test compound: S-23031, technical Description: light brown powder Batch #: PYG-88092-M Purity: 94.7% Contaminants: list in CBI appendix.

B. Test animals: Species: Rat Strain: Crl:CD BR Age: 12 weeks Weight (g): 325 - 430 (males), 216 - 259 (females) Source: Charles River Breeding Laboratories, Portage, MI.

II. STUDY DESIGN:

A. Animal assignment: Animals were assigned randomly to test groups as follows:

Table 1: Animal Assignment to Study Groups

Study Group	Dietary Level (ppm)	F_0 Generation ♂	F_0 Generation ♀	F_1 Generation ♂	F_1 Generation ♀
Control (CON)	0	10	10	10	10
Low (LDT)	1000	10	10	10	10
Mid1 (MDT1)	5000	10	10	10	10
Mid2 (MDT2)	10000	10	10	10	10
High (HDT)	20000	10	10	10	10

B. Diet Preparation: Appropriate amount of test compound was mixed with the basal diet (Purina Certified Rodent Chow #5002) to form a premix. The premix was then mixed with sufficient amount of basal diet to yield the correct concentration of test compound per dose group. The prepared diets were mixed to homogeneity (relative standard deviation of 0.41 to 2.9%) and were within 91.8 to 101 % of the target doses. Test diets were prepared weekly (diet mixtures were stable for at least 10 days). Animals in the control group received basal diet.

C. Study Design

1. Mating: The F_0 parental animals were given test diets for 28 days prior to mating. Approximately 16 week old animals were mated singly for a period of 10 days; vaginal smears were taken daily throughout the mating period. If there was no evidence of copulation after 10 days, the female was mated with another, known fertile, male for five additional days. If the second mating attempt was unsuccessful, the female was placed in a plastic cage with nesting material.

2. Selection and Rearing: On lactation day 4, the number of pups per litter was reduced to 10. Whenever possible, five pups of each sex were randomly selected; excess pups were weighed, sacrificed and discarded after the selection. All females were allowed to raise their pups to weaning (21 days) at which time the pups were sacrificed and necropsied.

D. Observation Schedule

1. F_0 Generation Animals: Observations and the schedule for those observations is summarized as follows:

Observation	Animals	Frequency
Mortality, moribundity and toxicity	All	Twice a day during premating and growth periods.
Detailed clinical observations	All	Once a week during growth and breeding periods.
Body weight	All	At beginning of study and weekly through growth and mating periods.
	Maternal	Gestation days 0, 7, 10, 14 and 20; lactation days 1, 4, 7, 14 and 21.
	Paternal	Weekly post-mating until sacrifice.
Food consumption	All	Weekly during premating period, on gestation days 0, 7, 10, 14 and 20 and on lactation days 1, 4, 7, 14 and 21.

2. Litter observations: Each litter was examined twice daily for survival. Determination of body weights and detailed clinical examinations were carried out on lactation days 1, 4, 7, 14 and 21. Pups were individually sexed on lactation days 0, 4 and 21.

E. Necropsy Examinations

1. Parental animals: Any F_0 animal found dead was subjected to a detailed postmortem examination. When breeding was completed, all F_0 males were sacrificed and subjected to a detailed postmortem examination. All females with viable pups were sacrificed on lactation day 21; females which did not deliver were sacrificed on day 25.

2. Offspring: The F_1 offspring were sacrificed on lactation day 21 and subjected to histopathological examination.

F. Data Analyses: Chi-square test, with Yates' correction factor, was used to determine the statistical significance of pup sex ratios, pup survival indices, mean number of stillborn and dead pups, and parental fertility indices. Two-tailed analysis of variance (ANOVA) and Dunnett's test were used to determine the statistical significance of F_0 body weights and weight gains, gestation and lactation body weights and weight gains, parental food consumption, mean litter weights, length of gestation, and live litter sizes.

G. Historical Control Data: Historical control data were provided to allow comparison with concurrent controls.

H. Quality Assurance: Quality assurance was documented by signed and dated GLP and quality assurance statements.

I. Flagging Statement: The sponsor applied the criteria of 40 CFR 158.34 for flagging studies for potential adverse effects to the results of this study. This study neither meets nor exceeds any of the applicable criteria.

II. RESULTS

A. F_0 Generation

1. Mortality and clinical signs: Two animals in the control group, one male and one female, were found dead on study week 5 and lactation day 20, respectively. All animals receiving the test compound survived until the scheduled sacrifice. The predominate, treatment-related clinical signs observed were the high incidence of tan and/or yellow matting and/or staining of the ano- and urogenital regions of both males and females in the 20000 ppm group and to a lesser extent in the 10000 ppm group.

2. Body Weight and Body Weight Gain: Males showed no treatment-related differences in either the mean weekly body weights or the mean body weight gain.

For females no treatment-related effects were observed on mean body weight. The mean body weight gain from week 0 to 2, however, was significantly decreased at the high dose only (Table 2). During the gestation period, no significant treatment-related effects on body weight and body weight gain were noted. The mean body weights and mean body weight gains during the lactation period showed significant increases in the 5000, 10000 and 20000 ppm groups.

3. Food Consumption:

a. Food consumption for males and females was not affected by treatment. Similarly, during the gestation and lactation periods, female food consumption was similar in the control and treatment groups.

b. Compound Consumption: Based on food consumption, body weight and dietary analyses, the compound consumption was calculated and summarized in Table 3, below.

4. Reproductive performance: No significant treatment-related effects were noted in any of the parameters measured to evaluate reproductive performance (Table 4).

5. Necropsy results: Gross examination of the of F_0 generation animals did not reveal any significant treatment-related effects.

Table 2: Body Weights and Body Weight Gain of Females

Observation	CON	LDT	MDT1	MDT2	HDT
Mean Body Weight (g)					
Lactation Day 14	307	324	334*	340**	330*
Mean Body Weight Gain (g)					
Premating Weeks 0 - 2	21	18	19	19	13*
Lactation Day 1 - 14	14	21	28	44**	42*

* $p \leq 0.05$, ** $p \leq 0.01$

Table 3: Mean Compound Consumption (mg/kg body weight/day)

Dietary Level (ppm)	Prior to Breeding		Gestation	Lactation
	Males	Females		
1000	61	72	76	153
5000	302	344	366	753
10000	617	707	765	1610
20000	1300	1450	1505	3055

B. F_1 Generation Results

1. Litter Data: The litter data for the F_1 generation pups, summarized in Table 5, were comparable between all of the study groups.

2. Necropsy results: Gross examination of the pups at the scheduled sacrifice did not reveal any significant, treatment-related findings.

Table 4: F_0 Generation Reproductive Performance

Observation	CON	LDT	MDT1	MDT2	HDT
Females on Study	10	10	10	10	10
Females that died	1	0	0	0	0
Females that delivered	9	10	10	10	10
Females, sacrificed day 25					
Gravid	0	0	0	0	0
Nongravid	1	0	0	0	0
Male Mating Index[a] (%)	100	80	90	90	100
Female Mating Index[b] (%)	100	90	100	100	100
Male Fertility Index[c]	90	100	100	100	100
Female Fertility Index[d]	90	100	100	100	100
Mean precoital interval (days)	2.2	3.4	4.3	3.6	2.0
Mean gestation interval (days)	21.9	22.0	21.8	21.6	21.9

a. Male Mating Index (%) = $\frac{\text{No. of Males with Evidence of Mating}}{\text{Total No. of Males Used for Mating}} \times 100$

b. Female Mating Index (%) = $\frac{\text{No. Females with Evidence of Mating}}{\text{Total no. of Females Used for Mating}} \times 100$

c. Male Fertility Index (%) = $\frac{\text{No. of Males Siring at Least 1 Litter}}{\text{Total No. of Males Used in Mating}} \times 100$

d. Female Fertility Index (%) = $\frac{\text{No. Females Pregnant}}{\text{Total No. Females Used in Mating}} \times 100$

Table 5: F_1 Generation Performance

Observation	CON	LDT	MDT1	MDT2	HDT
Number of litters	9[a]	10	10	10	10
Total litter losses	0	0	0	0	0
Before Selection[b]					
Mean live litter size (Day 1)	12.4	11.8	13.2	13.2	12.9
Mean live litter size (Day 4)	12.3	11.8	13.0	13.0	12.8
Number of pups (Day 1)	112	118	132	132	129
Number of pups (Day 4)	111	118	130	130	129
After Selection					
Mean live litter size (Day 1)	10.0	9.3	10.0	10.0	10.0
Mean live litter size (Day 4)	8.9	9.3	10.0	9.9	10.0
Number of pups (Day 4)	90	93	100	100	100
Number of pups (Day 21)	80	93	100	99	100
Pup deaths (Days 1-21)	1	0	2	3	0
M:F Sex Ratio (Day 0)	56:57	63:56	64:71	66:72	72:60
Mean pup weight (g) (Day 1)	6.8	7.2	6.8	6.7	6.7
Mean pup weight (g) (Day 21)	46.6	49.7	49.1	46.8	44.3

[a] One female in the control group died on lactation day 20, all of the pups were sacrificed.

[b] On lactation day 4, 10 pups were randomly selected from each litter, the remaining litter mates were sacrificed.

III. DISCUSSION

The effect of dietary administration of test compound, at dosages of 0, 1000, 5000, 10000 or 20000 ppm, was studied on the reproductive performance of rats through one generation. The incidence of adverse compound-related effects was low and was limited to tan matting and/or yellow matting or staining of the ano- and urogenital areas in male and female animals in the 10000 and 20000 ppm groups. Although there was a slight, but significant decrease in the maternal body weight gain during the first two weeks of the study, the effect was transient; during the breeding, gestation and lactation periods, both body weight and body weight gain were comparable between all of the study groups. The reproductive and litter performance parameters measured were also comparable between all of the study groups. No systemic or reproductive toxicity was evident at the highest dose of test compound studied (20000 ppm).

Conclusions: This range-finding study was designed to evaluate the reproductive and systemic toxicity of S-23031 administered to male and female rats for one generation. Dosages of 0, 1000, 5000, 10000 or 20000 ppm (equivalents to: 61, 302, 617 and 1300 mg/kg/day for males and 72, 344, 707 and 1450 mg/kg/day females, prior to breeding; 76, 366, 765 and 1505 mg/kg/day during gestation; 153, 753, 1610 and 3055 mg/kg/day during lactation) did not elicit significant systemic or reproductive toxicity.

	NOEL	LOEL
Systemic	20000 ppm	> 20000 ppm
Reproductive	20000 ppm	> 20000 ppm

Classification: core - Supplementary

This is not a guideline study.

000587

Reviewed by: Robert F. Fricke, Ph.D. Robert F. Fricke 19 June 92
Section IV, Tox. Branch II (H7509C)
Secondary Reviewer: Elizabeth A. Doyle, Ph.D. E. A. Doyle 6/29/92
Section IV, Tox. Branch II (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: Two generation Reproduction - Rat (83-4)

P.C. CODE: 128724

MRID NO.: 421698-35

TEST MATERIAL: S-23031

SYNONYMS: Pentyl 2-chloro-4-fluoro-5-[(3,4,5,6-tetrahydro) phthalimido] phenoxyacetate

STUDY NUMBER: WIL-118008

SPONSOR: Sumitomo Chemical Co., Ltd
Kitahama, 4-Chome 5-33, Chuo-Ku
Osaka, Japan

TESTING FACILITY: WIL Research Laboratories, Inc.
Ashland, OH 44805-9281

TITLE OF REPORT: A Dietary Two-Generation Reproduction Study of S-23031 in Rats

AUTHOR: M.D. Nemec

REPORT ISSUED: 10 May 1991

CONCLUSIONS: The effect of dietary administration of test compound, at dosages of 0, 200, 10000 or 20000 ppm (respective mg/kg/day equivalents are 16, 878, and 1715 (males) and 18, 829, and 1889 (females) prior to breeding; 14, 746 and 1551 during gestation; 32, 1670 and 3226 during lactation), was studied on the reproductive performance of rats over two generations. The systemic LOEL is based on increased absolute and relative liver and kidney weights at 10000 ppm. Reproductive LOEL is based on increased pup death on Day 0 in the 10000 ppm group.

	NOEL	LOEL
Systemic	200 ppm	10000 ppm
Reproductive	200 ppm	10000 ppm

CLASSIFICATION: core - Guideline

This study satisfies the guideline (83-4) for reproductive and fertility effects in rats.

I. MATERIALS:

A. Test compound: S-23031, technical Description: light brown powder Batch #: PYG-88092-M Purity: 94.7% Contaminants: not given

B. Test animals: Species: Rat Strain: Crl:CD BR Age: 38 days Weight (g): 130 - 189 (males), 110 - 146 (females) Source: Charles River Breeding Laboratories, Portage, MI.

II. STUDY DESIGN:

A. Animal assignment: Animals were assigned randomly to study groups as follows:

Table 1: Animal Assignment to Study Groups

Study Group	Dietary Level (ppm)	Number of Animals[a] Male	Female
Control (CON)	0	30	30
Low (LDT)	200	30	30
Mid (MDT)	10000	30	30
High (HDT)	20000	30	30

[a] Number of parental animals in the F_0 and F_1 generations

B. Diet Preparation: An appropriate amount of test compound was mixed with the basal diet (Purina Certified Rodent Chow #5002) to form a premix. The premix was then mixed with sufficient amount of basal diet to yield the correct concentration of test compound per dose group. The prepared diets were mixed to homogeneity; no significant differences were found in samples taken from the top, middle and bottom of the test diets (relative standard deviations of 1.84, 1.84, and 0.75% for LDT, MDT and HDT, respectively). Test diets were also within 95.0 and 100% of the target concentration. Test diets were prepared weekly and were stable for at least 10 days.

C. Study Design (see Appendix 1)

1. Mating: The F_0 generation parental animals were approximately 16 weeks old when mated and had been given test diet for 74 days. Within each treatment group, a single male was mated with a single female for a period of 10 days; vaginal smears were taken daily throughout the mating period. If, after 10 days there was no evidence of copulation, the female was mated

with another, known fertile, male for five additional days. Any female, not showing evidence of mating, was placed in a plastic cage with nesting material. If pups were not delivered after 25 days, these females were sacrificed.

The F_1 generation parental animals were 16 weeks old when mated and had been exposed to the test diet for 81 days. The mating procedure was identical to that outlined above for the F_0 generation animals.

2. Litter Reduction and Rearing: On lactation day 4, the number of pups in both the F_0 and F_1 generations was reduced to 10/litter. Whenever possible, five animals of each sex were randomly selected for assessment of growth and survival. All females in each dose group were allowed to raise their pups to weaning (21 days).

D. Observation Schedule

1. F_0 and F_1 Generation Parental Animals: Observations and the schedule for those observations are summarized as follows:

Observation	Animals	Frequency
Mortality, moribundity and toxicity	All	Twice a day during premating and growth periods.
Detailed clinical observations	All	Once a week during growth and breeding periods.
Body weight	All	At beginning of study and weekly through growth and mating periods.
	Maternal	Gestation days 0, 7, 10, 14 and 20; lactation days 1, 4, 7, 14, and 21.
	Paternal	Weekly post-mating until sacrifice.
Food consumption	All	Weekly during premating period, on gestation days 0, 7, 10, 14 and 20, and on lactation days 1, 4, 7, 14 and 21.

2. F_1 and F_2 Generation Litter Observations: Each litter was examined twice daily for survival. Determination of body weights and detailed clinical examinations were carried out on lactation days 1, 4, 7, 14 and 21. Pups were individually sexed on lactation days 0, 4 and 21.

E. Sacrifice and Pathology: Any parental animal found dead was subjected to a detailed postmortem examination. Surviving F_0 and F_1 generation parental animals were sacrificed approximately five weeks and two weeks after weaning, respectively, and subjected to a detailed postmortem examination. On lactation day 21 five F_1 weanlings/group/sex were randomly selected and subjected to necropsy examination. F_2 weanlings were necropsied on lactation day 21.

The checked (X) tissues were collected at time of necropsy. For the F_0 parental animals and five selected F_1 and F_2 weanlings/sex/group the checked (XX) organs were also weighed. In addition to the tissues marked XX, the epididymides, prostate, seminal vesicles, uterus and brain were also weighed for the F_1 parental animals. Liver, kidneys, epididymides, prostate, seminal vesicle, ovaries, uterus, vagina, pituitary, and gross lesions from all F_0 and F_1 parental animals and the selected F_1 and F_2 weanlings in the control and 20000 ppm groups were examined histologically. The kidneys from the 200 and 10000 ppm groups were also examined histologically.

Digestive system		**Cardiovas./Hematol**		**Neurologic**	
	Tongue	X	Aorta	X	Brain
X	Salivary glands	X	Heart	X	Periph. nerve
X	Esophagus	X	Bone marrow	X	Spinal cord
X	Stomach	X	Lymph nodes	XX	Pituitary
X	Duodenum	X	Spleen	X	Eyes
X	Jejunum	X	Thymus	**Glandular**	
X	Ileum	**Urogenital**		X	Adrenals
X	Cecum	XX	Kidneys		Lacrimal gland
X	Colon	X	Urinary bladder	X	Mammary gland
X	Rectum	XX	Testes		Parathyroids
XX	Liver	X	Epididymides	X	Thyroids
	Gall bladder	X	Prostate	**Other**	
X	Pancreas	X	Seminal vesicle	X	Bone
Respiratory		XX	Ovaries	X	Skeletal muscle
X	Trachea	X	Uterus	X	Skin
X	Lungs	X	Vagina	X	Gross lesions
	Nasal Passages	X	Cervix		

F. Data Analyses: Chi-square test with Yates' correction factor was used to determine the statistical significance of pup sex ratios, pup survival indices, mean number of stillborn and dead pups, and parental fertility indices. ANOVA (two-tailed) and Dunnett's test were used to determine the statistical significance of parental body weights and weight gains, gestation and lactation body weights and weight gains, parental food consumption, mean litter weights, length of gestation, and live litter sizes. The Kolmogorov-Smirnov test (one-tailed) was used to determine the statistical significance of the histological findings.

G. Quality assurance was documented by signed and dated GLP and quality assurance statements.

H. The sponsor applied the criteria of 40 CFR 158.34 for flagging studies for potential adverse effects to the results of this study. This study neither meets nor exceeds any of the applicable criteria.

II. RESULTS

A. F_0 and F_1 Parental Data

1. Mortality and Clinical Signs: With the exception of one male and one female in the control group, which were found dead on study week 5 and lactation day 21, respectively, all animals survived until the scheduled sacrifice.

The predominant clinical signs for both the F_0 and F_1 parental animals were an increase in the incidence of tan and/or yellow matting or staining of the uro- and anogenital areas and brown staining on the tail of animals in the 10000 and 20000 ppm groups.

2. Body Weight and Body Weight Gain: The effect of treatment on weekly body weights is summarized in Table 2, below. The F_0 and F_1 males in the 20000 ppm group showed significant decreases in the mean body weight. The body weights of the F_0 females were not affected by treatment. The F_1 females in the 20000 ppm group had significant decreases in body weight throughout the study; decreased body weight in the 10000 ppm group was infrequent.

Mean body weight gain for the F_0 and F_1 parental animals is summarized in Table 3. For F_0 and F_1 males and F_0 females, no clear treatment-related on body weight gain were noted. The F_1 females showed a significant decrease only during week 18 to 19. The cumulative body weight gains from week 0 for the F_0 generation and from week 19 for the F_1 generation are summarized in Table 4, below. Significant decreases in the cumulative body weight gains were found only in the F_0 and F_1 males in the 20000 ppm group.

The mean body weights during the gestation and lactation periods are summarized in Table 5. No treatment-related effects on mean body weights were observed in the F_0 females. However, values for the F_1 animals were significantly lower in the 20000 ppm group during most of the gestation period and the first day of the lactation period. Increased mean body weight gain (Table 6) was observed in the F_1 females during gestation and F_2 and F_1 females on days 1 to 4 of lactation period 10000 and 20000 ppm groups

Table 2: Mean Body Weight (g) for F_0 and F_1 Parental Animals (Data summarized from Tables 4 and 40 of study)

	Week of Study	CON	LDT	MDT	HDT
F_0 - Males	5	363	368	367	345*
	6	388	391	390	369*
	11	466	463	462	438*
	13	498	499	495	472*
	14	510	506	503	474*
	15	524	517	518	495*
F_1 - Males	20	178	179	173	155*
	21	231	234	229	208*
	22	281	283	281	255**
	23	326	328	323	295**
	24	359	361	355	321**
	25	387	392	384	348**
	26	414	418	408	372**
	27	438	441	432	397**
	28	458	462	453	418**
	29	473	479	471	441*
F_1 - Females	18	72	73	72	65**
	19	105	109	100	90*
	20	138	139	131	122**
	21	164	160	156	147**
	22	186	182	177	165**
	23	203	199	194	183**
	24	216	213	204*	193**
	25	229	227	215*	207**
	26	240	240	226*	216**
	27	247	246	234	223**
	28	255	251	239*	230**
	29	260	260	245*	235**
	38	298	296	290	281*
	39	303	303	295	281**
	40	309	302	292	285*

* $p \leq 0.05$, ** $p \leq 0.01$

Table 3: Mean Body Weight Gain (g) for F_0 and F_1 Parental Animals (Data summarized from Tables 5 and 41 of study)

	Week of Study	CON	LDT	MDT	HDT
F_0 - Males	2 - 3	37	39	36	26**
	11 - 12	13	14	15	19**
	15 - 16	9	11	10	13*
	17 - 18	7	11	11	13*
	18 - 19	8	5	5	2**
F_0 - Females	2 - 3	15	16	19*	18
	7 - 8	11	8	10	7*
	8 - 9	6	8	-6**	2
	9 - 10	3	5	14**	9**
F_1 - Males	18 - 19	50	51	49	41**
	19 - 20	59	57	55	47**
	23 - 24	33	34	32	26**
	28 - 29	15	18	18	23**
	29 - 30	8	8	11	14*
	38 - 39	6	3	-16**	-7**
	39 - 40	9	9	26**	23**
F_1 - Females	18 - 19	41	39	35*	32**

* $p \leq 0.05$, ** $p \leq 0.01$

Table 4: Cumulative Mean Body Weight Gain (g) for F_0 and F_1 Males (Data summarized from Tables 6 and 42 of study)

	Week of Study	CON	LDT	MDT	HDT
F_0 - Males	0 - 2	109	110	117**	109
	0 - 3	146	148	154	136*
	0 - 4	179	183	187	165*
	0 - 5	206	211	211	188**
	0 - 6	231	234	233	212*
	0 - 7	250	255	254	233*
	0 - 8	269	272	270	249*
	0 - 9	288	290	285	266*
	0 - 10	303	304	301	281*
	0 - 11	309	306	305	281**
	0 - 12	322	321	320	300*
	0 - 13	341	342	339	315*
	0 - 14	353	349	346	317**
	0 - 15	367	360	361	338*
F_1 - Males	19 - 20	59	57	55	47**
	19 - 21	112	112	111	100**
	19 - 22	163	162	163	147**
	19 - 23	207	206	205	186**
	19 - 24	240	240	237	213**
	19 - 25	268	271	266	240**
	19 - 26	295	296	289	264**
	19 - 27	319	320	314	288**
	19 - 28	340	340	335	310*

* $p \leq 0.05$, ** $p \leq 0.01$

Table 5: Mean Body Weights (g) of F_1 Females During Gestation and Lactation (Data summarized from Tables 43 and 45 of study).

	Day	CON	LDT	MDT	HDT
F_1: Gestation	0	262	261	249	237**
	7	291	287	279	265**
	10	301	298	290	279**
	14	318	314	306	295**
F_1: Lactation	1	290	286	282	270*

* $p \leq 0.05$, ** $p \leq 0.01$

Table 6: Mean Body Weight Gain (g) of F_0 and F_1 Females During Gestation and Lactation (Data summarized from Tables 8, 10 and 46 of study)

	Days	CON	LDT	MDT	HDT
F_0: Gestation	0 - 10	35	37	42	44*
	0 - 14	53	58	63*	64**
Lactation	1 - 4	10	9	16	19**
	4 - 7	-1	6	-4	-12*
F_1: Lactation	1 - 4	4	10	13*	15**
	1 - 7	12	13	25**	26**
	1 - 14	19	26	40**	39**
	1 - 21	1	3	11	17*

* $p \leq 0.05$, ** $p \leq 0.01$

3. Food and Compound Consumption:

a. Food Consumption: Food consumption (g/animal/day) by F_0 and F_1 males and females is summarized in Table 7. Significant differences were observed, however, the magnitude of the responses was too small to be biologically meaningful.

Food consumption results (g/kg body weight/day) for the F_0 and F_1 males and female parental animals are presented in Table 8 (Note: Food efficiency, expressed as grams food consumed/100 gram change in body weight/day, was not calculated in the study). Significant increases in food consumption were observed in males and females of both generations. Significant findings were reported in the 20000 ppm group with infrequent significant findings observed in the 10000 ppm group.

Food consumption during the gestation and lactation periods is summarized in Table 9, below. For the F_1 females, no treatment-related effects were noted during lactation; for gestation days 7 to 10, food consumption was significantly higher only in the high dose group. Significant increases were noted in F_1 females during gestation and lactation and were limited to the 20000 ppm group.

b. Compound Consumption: Based on food consumption, body weight and dietary analyses, the compound consumption was calculated and summarized in Table 10 below.

Table 7: Food Consumption (g/animal/day) for F_0 and F_1 Parental Animals (Data summarized from Tables 11 and 47 of study)

	Week of Study	CON	LDT	MDT	HDT
F_0 - Males	14 - 15	27	26	28	29**
	15 - 16	27	26	27	28*
	17 - 18	25	25	26	28**
	21 - 22	25	25	25	27*
F_0 - Females	7 - 8	19	19	19	20*
	9 - 10	19	19	20	20**
	20 - 21	19	20	20	21**
	21 - 22	19	18	20	21**
	22 - 23	17	19	19	19*
F_1 - Males	18 - 19	16	17	16	14**
	19 - 20	20	21	20	18**
	20 - 21	24	23	23	22*
	21 - 22	25	25	25	24*
	23 - 24	25	24	25	23**
	29 - 30	25	25	27	28*
F_1 - Females	18 - 19	14	15	13	13*
	19 - 20	17	16	15**	14**
	20 - 21	17	17	16	15**
	21 - 22	18	17	17	16**
	22 - 23	18	18	17	16**
	23 - 24	18	18	16*	15**
	24 - 25	20	20	18*	18*

* $p \leq 0.05$, ** $p \leq 0.01$

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Table 8: Food Consumption (g/kg body weight/day) for F_0 and F_1 Parental Animals (Data summarized from Tables 12 and 48 of study)

	Week of Study	CON	LDT	MDT	HDT
F_0 - Males	3 - 4	79	78	81	83**
	4 - 5	77	78	77	83**
	5 - 6	71	73	74	78**
	6 - 7	64	65	66	70**
	7 - 8	63	63	63	67**
	8 - 9	60	59	59	63*
	9 - 10	58	58	60	63**
	10 - 11	58	59	58	61*
	14 - 15	52	51	55*	61**
	15 - 16	50	50	52	56**
	16 - 17	49	50	51	53**
	17 - 18	46	46	49	53**
	18 - 19	47	46	48	50**
	19 - 20	45	45	47	51**
	20 - 21	44	45	45	48**
	21 - 22	44	44	44	50**
F_0 - Females	7 - 8	77	78	79	82**
	9 - 10	73	74	80**	80**
	20 - 21	62	66	67	71**
	21 - 22	62	62	64	68*
	22 - 23	58	64**	60	63*
F_1 - Males	24 - 25	72	71	74	80**
	25 - 26	67	66	71	76**
	26 - 27	64	64	65	70**
	27 - 28	61	59	63	67**
	28 - 29	58	55	60	64**
	29 - 30	53	52	56	61**
	32 - 33	50	48	52	55**
	34 - 35	50	48	52	55*
	38 - 39	45	46	45	50*
	39 - 40	47	45	47	54**
F_1 - Females	25 - 26	80	80	84	86*

* $p \leq 0.05$, ** $p \leq 0.01$

Table 9: Food Consumption (g/kg body weight/day) for F_0 and F_1 Animals During Gestation and Lactation (Data summarized from Tables 14, 50 and 52 of study)

	Day of Study	CON	LDT	MDT	HDT
F_0 - Gestation	7 - 10	77	80	80	84*
F_1 - Gestation	0 - 7	75	74	78	81*
	7 - 10	75	73	77	82*
	10 - 14	71	71	76	82**
	0 - 10	75	73	77	80*
	0 - 14	73	72	76	80**
	0 - 20	69	67	71	75**
F_1 - Lactation	1 - 14	139	146	156	157*
	1 - 21	173	175	192**	178

* $p \leq 0.05$, ** $p \leq 0.01$

Table 10: Mean Compound Consumption (mg/kg body weight/day) (Data summarized from Tables 17, 18, 19, 53, 54, and 55 of study)

Dietary Level (ppm)	Prior to Breeding Males	Prior to Breeding Females	Gestation	Lactation
F_0 Generation				
200	16	18	15	33
10000	781	925	764	1625
20000	1610	1869	1553	3197
F_1 Generation				
200	17	19	14	31
10000	878	936	729	1716
20000	1821	1909	1550	3256
Average for F_0 and F_1 Generations				
200	16	18	14	32
10000	878	829	746	1670
20000	1715	1889	1551	3226

4. Reproductive performance: The reproductive performance and indices (listed below) of the F_0 and F_1 animals are summarized in Table 11, below. No significant treatment-related effects were noted in any of the parameters measured to evaluate reproductive performance.

$$\text{Male Mating Index (\%)} = \frac{\text{No. of Males with Evidence of Mating}}{\text{Total No. of Males Used for Mating}} \times 100$$

$$\text{Female Mating Index (\%)} = \frac{\text{No. females with Evidence of Mating}}{\text{Total no. of Females Used for Mating}} \times 100$$

$$\text{Male Fertility Index (\%)} = \frac{\text{No. of Males Siring at Least 1 Litter}}{\text{Total No. of Males Used in Mating}} \times 100$$

$$\text{Female Fertility Index (\%)} = \frac{\text{No. Females Pregnant}}{\text{Total No. Females Used in Mating}} \times 100$$

$$\text{Female Pregnancy Index (\%)} = \frac{\text{Total No. of Pregnant Females}}{\text{No. of Females Mated}} \times 100$$

5. Organ Weight Results: The organ weight data for the F_0 and F_1 parental animals are summarized in Table 12, below. With the exception of the absolute organ weights of the F_0 males and F_1 females, both the absolute and relative kidney and liver weights were significantly increased in the 10000 and 20000 ppm groups. The F_0 males and F_1 females showed significant increases in the absolute liver and kidney weights only, respectively.

6. Necropsy results

a. Gross Pathology: Although the incidence of gross pathological observations for the treated animals was not significantly different from the control values, there appears to be a dose-related increase in the incidence of yellow matting in the females and brown staining in both the F_0 males and females (Table 13). Incidence data for the F_1 parental animals did not show any significant or dose-related effects.

b. Histopathology: Histopathology data are presented in Table 14. The only significant finding was the increased incidence of minimal nephropathy in F_1 males. A dose-related, but insignificant, increase in the incidence of nephropathy in F_0 and F_1 males and F_1 females was observed. The incidence of mild hyperkeratosis increased in a dose-dependent manner in the F_0 males and females.

Table 11: F_0 and F_1 Reproductive Performance (Data summarized from Tables 2, 38, 20 and 56 of the study)

Observation	CON	LDT	MDT	HDT
F_0 Generation				
Females on Study	30	30	30	30
Females that died	1	0	0	0
Gravid	0	0	0	0
Nongravid	1	0	0	0
Females Allowed to Deliver	30	30	30	30
Nongravid	3	3	2	4
Gravid	27	27	28	26
Delivered				
With Live Pups	27	26[a]	28	26
With Total Litter Loss	1	0	0	0
Male Mating Index (%)	86.7	93.3	86.7	83.3
Female Mating Index (%)	96.7	100	93.3	93.3
Male Fertility Index (%)	92.3	92.9	100	96.0
Female Fertility Index (%)	90.0	90.0	93.3	66.7
Female Pregnancy Index (%)	93.1	90.0	100	92.9
Mean precoital interval (days)	3.9	3.7	3.6	4.1
Mean gestation interval (days)	21.8	21.7	21.8	21.8
F_1 Generation				
Females on Study	30	30	30	30
Females that died	0	0	0	0
Gravid	0	0	0	0
Nongravid	0	0	0	0
Females Allowed to Deliver	30	30	30	30
Nongravid	2	0	1	3
Gravid	28	30	29	27
Delivered				
With Live Pups	28	30	29	27
With Total Litter Loss	0	0	0	0
Male Mating Index (%)	96.7	100	90.0	93.3
Female Mating Index (%)	96.7	100	100	96.7
Male Fertility Index (%)	96.6	100	96.3	92.9
Female Fertility Index (%)	93.3	100	96.7	90.0
Female Pregnancy Index (%)	96.6	100	96.7	93.1
Mean precoital interval (days)	2.9	2.4	3.0	3.3
Mean gestation interval (days)	21.8	21.6	21.7	21.7

[a] One female was gravid (1 implantation site) but there was no evidence of delivery.

Table 12: Absolute and Relative Organ Weights for Parental Animals (Data summarized from Tables 33, 34, 68, 69, and 69A of the study)

Observation	Organ	CON	LDT	MDT	HDT
Absolute Organ Weights (g)					
F_0 - Males	Liver	19.84	20.28	21.05	23.64**
Females	Kidneys	2.07	2.22*	2.28**	2.35**
	Kidneys	2.07	2.19[a]	2.28**	2.35**
	Liver	10.33	10.57	11.40*	12.34**
F_1 - Males	Kidneys	3.72	3.91	4.12**	4.48**
	Liver	19.96	20.58	22.93**	25.75**
Females	Kidneys	2.19	2.25	2.45**	2.50**
Organ Weight Relative to Body Weight (g/100 g)					
F_0 - Males	Kidneys	0.659	0.669	0.689	0.731**
	Liver	3.463	3.552	3.712*	4.274**
Females	Kidneys	0.690	0.732[a]	0.749*	0.772**
	Liver	3.436	3.534	3.724**	4.019**
F_1 - Males	Kidneys	0.635	0.662	0.714**	0.783**
	Liver	3.391	3.473	3.935**	4.461**
Females	Kidneys	0.710	0.739	0.816**	0.877**
	Liver	3.590	3.745	3.953**	4.192**
	Brain	0.651	0.664	0.682	0.695*
Organ Weight Relative to Brain Weight (g/100 g)					
F_1 - Males	Kidneys	171	181	193**	207**
	Liver	915	952	1070**	1188**
F_1 - Females	Kidneys	110	112	120**	127**
	Liver	556	571	584	611*

[a] The kidney weight for one animal was excluded from the statistical evaluations since it was an apparent outlier. The value (3.13 g) was approximately 3.7 standard deviations higher than the mean kidney weight for this group.
* $p \leq 0.05$, ** $p \leq 0.01$

Table 13: Incidence of Gross Pathology Findings for F_0 Parental Animals (Data summarized from Table 31 of the study)

	Males				Females			
Observation	CON	LDT	MDT	HDT	CON	LDT	MDT	HDT
Number Examined	30	30	30	30	29	30	30	30
Yellow uro- and/or anogenital matting	0	0	1	2	0	0	2	13
Brown staining on tail	0	1	10	21	0	0	10	23

Table 14: Histopathology Findings for F_0 and F_1 Parental Animals (Data summarized from Tables 36 and 70 of the study)

	Males				Females			
Observation	CON	LDT	MDT	HDT	CON	LDT	MDT	HDT
F_0 Generation								
Number Examined	30	30	30	30	29	30	30	30
Nephropathy	4	5	13	13	1	0	0	0
Minimal	2	3	9	11	1	0	0	0
Mild	1	2	3	2	0	0	0	0
Moderate	0	0	1	0	0	0	0	0
Severe	1	0	0	0	0	0	0	0
Hyperkeratosis	NA	NA	NA	20	NA	NA	NA	16
Minimal	NA	NA	NA	5	NA	NA	NA	4
Mild	NA	NA	NA	14	NA	NA	NA	12
Moderate	NA	NA	NA	1	NA	NA	NA	NA
F_1 Generation								
Number Examined	30	30	30	30	30	30	30	30
Nephropathy	3	4	7	17*	NA	NA	NA	16
Minimal	2	3	7	11	NA	NA	NA	4
Mild	1	1	none	6	NA	NA	NA	12
Hyperkeratosis	NA	NA	NA	6	NA	NA	NA	2
Minimal	NA	NA	NA	1	NA	NA	NA	1
Mild	NA	NA	NA	5	NA	NA	NA	1

NA - not applicable
* $p \leq 0.05$

B. F_1 and F_2 Litter Results

1. Litter Data: The litter data for the F_1 and F_2 generations are summarized in Table 15. The number of pup deaths on Day 0 was significantly increased in the 10000 and 20000 ppm groups. Further, the sex ratio of the F_1 pups in the 20000 ppm group showed a significantly higher number of males. A secondary effect of the increased number of male pups was a significant increase in the mean pup body weight on Day 1. The mean body weight for the F_2 pups was significantly lower in the 20000 ppm group.

2. Organ Weights: Organ weight data for the F_1 and F_2 pups are summarized in Table 16, below. Significant findings were limited to the kidneys and liver. The sponsor indicated that one of the kidney weights, 3.13 g, in the 200 ppm group was higher in one animal. Inclusion of this value in the statistical analysis resulted in significant increase in the 200 ppm group. Since the value in question is approximately 3.7 standard deviations higher than the mean kidney weight for this group, it must be considered an outlier and was excluded from the statistical evaluations. If excluded, the mean kidney weight for the 200 ppm group is no longer significantly different.

3. Necropsy Results:

a. Gross Pathology: At the scheduled sacrifice on lactation day 21, the incidence of gross pathological lesions in the F_1 and F_2 weanlings was comparable among all of the study groups.

b. Histopathology: No significant differences were found in any of the tissues subjected to examination.

III. DISCUSSION

The effect of dietary administration of test compound, at dosages of 0, 200, 10000 or 20000 ppm, was studied on the reproductive performance of rats over two generations. No treatment-related lethalities occurred in the study. The predominant clinical signs for both the F_0 and F_1 parental animals were an increase in the incidence of tan and/or yellow matting or staining of the uro- and anogenital areas and brown staining on the tail of animals in the 10000 and 20000 ppm groups.

At 20000 ppm the mean body weights were decreased; at 10000 ppm occasional decreases in body weights were noted. No clear treatment-related effects were noted on the mean body weight gain, where significant increases and decreases occurred. The cumulative body weight gains were consistently increased in the males in the 20000 ppm group.

Table 15: F_1 and F_2 Pup Viability Data (Data summarized from Tables 21, 24, 57 and 60 of the study)

Observation	CON	LDT	MDT	HDT
F_1 Generation Animals				
Number of Litters	27	26	28	26
Total Litter Losses	1	0	0	0
Mean Live Litter Size - Day 0	12.6	13.2	12.3	11.9
- Day 1	12.4	13.0	12.0	11.7
- Day 4	12.3	12.8	12.0	11.7
Number of Pups - Day 0	341	343	344	309
- Day 1	336	338	337	305
- Day 4	333	334	335	303
- Day 4[a]	253	258	268	249
- Day 21	252	258	267	246
Pup Deaths - Day 0	4	5	15*	16**
- Days 1 to 4	3	4	2	6
- Days 4 to 21	1	0	1	3
Sex Ratio (M:F)	162:179	170:173	182:162	174:135*
Mean Pup Weight (g) - Day 1	6.5	6.5	6.7	7.0*
- Day 21	44.4	45.5	46.8	43.8
F_2 Generation Animals				
Number of Litters	28	30	29	27
Total Litter Losses	0	0	0	0
Mean Live Litter Size - Day 0	13.0	13.3	12.7	12.9
- Day 1	12.4	13.0	12.0	11.7
- Day 4	12.3	12.8	12.0	11.7
Number of Pups - Day 0	365	398	367	347
- Day 1	356	394	364	341
- Day 4	355	389	360	339
- Day 4[a]	274	296	289	263
- Day 21	273	294	289	257
Pup Deaths - Day 0	8	7	9	6
- Days 1 to 4	1	5	4	2
- Days 4 to 21	1	2	0	6
Sex Ratic (M:F)	167:198	180:218	174:193	176:171
Mean Pup Weight (g) - Day 1	6.6	6.5	6.6	6.6
- Day 21	45.2	46.9	45.4	41.6**

[a] After selection of 5 pups/sex/litter

* $p \leq 0.05$, ** $p \leq 0.01$

Table 16 F_1 and F_2 Absolute and Relative Organ Weights for Pups Sacrificed on Lactation Day 21 (Data summarized from Tables 27, 28, 63 and 64 of the study)

Observation	Organ	CON	LDT	MDT	HDT
Absolute Organ Weights g.					
F_1 - Females	Kidneys	0.51	0.60	0.65**	0.59
F_2 - Males	Liver	1.55	1.96	2.11*	2.05*
	Kidneys	0.48	0.54	0.59*	0.57
Organ Weight Relative to Body Weight (g/100 g)					
F_1 - Males	Kidneys	1.187	1.215	1.297	1.397*
F_2 - Males	Kidneys	1.113	1.135	1.312	1.359*
	Liver	3.598	4.039	4.658**	4.873**
F_2 - Females	Kidneys	1.259	1.220	1.325	1.516**

* $p \leq 0.05$, ** $p \leq 0.01$

No differences were noted in the reproductive performance of the F_0 and F_1 generation animals. However, there was a significantly higher rate of F_1 pup deaths on Day 0 in both the 10000 and 20000 ppm groups. This observation was judged to be a significant reproductive effect. The sex ratio was significantly higher in the 20000 ppm group. The higher number of male pups resulted in a significant increase in the mean pup body weight in the 20000 ppm group. The only other significant finding was a significantly lower mean pup body weight for the F_2 generation pups in the 20000 group on Day 21.

Significant increases in the absolute and relative liver and kidney weights were noted in the F_0 and F_1 generation parents and the F_1 and F_2 generation pups in the 10000 and 20000 ppm groups. For male animals histopathological examination showed an increase (but not statistically significant) in the incidence of nephropathy; for the F_1 generation males the incidence was significantly increased in the 20000 ppm group.

III. CONCLUSIONS

The effect of dietary administration of test compound, at dosages of 0, 200, 10000 or 20000 ppm (respective mg/kg/day equivalents are 16, 878, and 1715 (males) and 18, 829, and 1889 (females) prior to breeding; 14, 746 and 1551 during gestation; 32, 1670 and 3226 during lactation), was studied on the reproductive performance of rats over two generations. The systemic LOEL is based on increased absolute and relative liver and kidney weights at 10000 ppm. Reproductive LOEL is based on increased pup deaths on Day 0 in the 10000 ppm group.

	NOEL	LOEL
Systemic	200 ppm	10000 ppm
Reproductive	200 ppm	10000 ppm

Classification: core - Guideline

This study satisfies the guideline (83-4) for reproductive and fertility effects in rats.

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Reviewed by: Robert F. Fricke, Ph.D. Robert F. Fricke 5 May 92
Section IV, Tox. Branch II (H7509C)
Secondary Reviewer: Elizabeth A. Doyle, Ph.D. E. A. Doyle 5/5/92
Section IV, Tox. Branch II (H7509C)

DATA EVALUATION REPORT

STUDY TYPE:	Salmonella/mammalian activation gene mutation assay (84-2)
P.C. CODE:	128724
MRID NO.:	421698-36
TEST MATERIAL:	S-23031
SYNONYMS:	Pentyl 2-chloro-4-fluoro-5-[(3,4,5,6-tetrahydro)phthalimido] phenoxyacetate
STUDY NUMBER:	1677
SPONSOR:	Sumitomo Chemical Co., Ltd. Kitahama, 4-Chome 5-33, Chou-Ku Osaka, Japan
TESTING FACILITY:	Sumitomo Chemical Co., Ltd. Biochemistry and Toxicology Laboratory 1-98, 3-Chome Kasugade-naka, Konohana-ku Osaka, Japan
TITLE OF REPORT:	Reverse Mutation Test of S-23031 in Salmonella typhimurium and Escherichia coli
AUTHOR:	S. Kogiso
REPORT ISSUED:	22 August 1989
CONCLUSIONS:	The test compound, at doses of 100, 200, 500, 1000, 2000, and 5000 μg/plate, was not mutagenic in the assay either with of without S-9 activation.
CLASSIFICATION:	Core - unacceptable

(May be upgraded to acceptable if information is provided to indicate that the tester strains were properly maintained and that they were checked for genetic markers.)

This study does not satisfy the guideline (84-2) requiements for a "Gene Mutation".

A. **MATERIALS**

1. Test compound: S-23031, technical Description: brown powder
Batch #: PYG-88092-M Purity: 94.4% Contaminants: Not given.

2. Control Materials

Negative control: Vehicle (DMSO)

Final concentration of solvent : 100 µl/plate

Positive Controls:

Without Activation:

Strain	µg/plate	Positive Control
TA98	0.1	2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (AF-2)
TA100	0.01	"
TA1535	0.5	Sodium azide
TA1537	80	9-Aminoacridine
TA1538	2	2-Nitrofluorene
WP2uvrA	2	N-ethyl-N'-nitro-N-nitrosoguanidine

With Activation:

Strain	µg/plate	Positive Control
TA98	5	Benzo(a)pyrene
TA100	"	"
TA1537	"	"
TA1538	"	"
TA1535	2	2-Aminoanthracene
WP2uvrA	20	"

3. Activation: S-9 derived from PCB (Kenachlor-400) -induced rat liver. The S-9 fraction was stored at -80°C until used.

One ml of S-9 mixture contained 0.2 ml water, 0.1 ml 80 mM $MgCl_2$, 0.1 ml 330 mM KCl, 1.7 mg glucose-6-phosphate, 3.62 mg NADPH, 3.05 mg NADH, 0.5 ml 200 mM sodium phosphate buffer (pH 7.4), and 0.1 ml S-9 fraction. The S-9 mixture was prepared fresh, before each use.

4. Test Organisms: TA98, TA100, TA1535, TA1537, TA1538, WP2uryA

There is no indication in the study that the tester strains were properly maintained or that they were checked for appropriate genetic markers (rfa mutation, R factor).

5. Test Compound Concentrations Used:

Non-activated: 5000, 2000, 1000, 500, 200, and 100 µg/plate
Activated: 5000, 2000, 1000, 500, 200, and 100 µg/plate

B. TEST PERFORMANCE

1. Type of Salmonella Assay: Preincubation method (20 minutes). References: Ames *et al.*, Mutation Res. 31, 347-364 (1975), Maron and Ames, Mutation Res. 113, 173-215 (1983), Yahagi *et al.* Cancer Letters 1, 91-96 (1975).

Protocol: Six dose levels were selected for inclusion in the study on the basis of a preliminary range-finding study which showed no cytotoxicity of test compound up to 5000 µg/plate. All dose levels were plated in duplicate for both non-activated and activated conditions; results were confirmed in a duplicate assay.

The test compound solution (0.1 ml), test bacteria suspension (0.1 ml) and either 100 mM sodium phosphate buffer (0/5 ml, pH 7.4) or S-9 mix (0.5 ml) were mixed in a small test tube and incubated, with shaking, at 37°C for 20 minutes. After addition of 2 ml of a melted top agar, the mixture was poured onto a minimal glucose agar plate. After a 65 hr-incubation at 37°C, the revertant colonies on the plate were counted using an automatic colony counter.

The criteria for determination of mutagenic potential were as follows: The number of revertant colonies induced by the test compound must be twice that of the vehicle control and there must be a dose-dependent increase in the number of revertant colonies.

2. Preliminary Cytotoxicity Assay: No evidence of cytotoxicity was reported at doses up to 5000 µg/plate. Precipitation of the test compound in the medium occurred at dose levels ≥ 1000 µg/plate without activation and at 5000 µg/plate with activation. Therefore, 5000 µg/plate was selected as the practical upper dosing limit due to the limits of solubility.

3. Mutagenicity Assay: No positive responses were reported for the test compound either with or without S-9 activation.

4. Reviewer's Discussion/Conclusions: The test compound, at doses of 100, 200, 500, 1000, 2000, and 5000 µg/plate, was not mutagenic in the assay either with of without S-9 activation.

Classification: core - unacceptable

(May be upgraded to acceptable if information is provided to indicate that the tester strains were properly maintained and that they were checked for genetic markers.)

5. The study was performed under GLP's; a signed and dated GLP compliance statement is present.

Reviewed by: Robert F. Fricke, Ph.D. Robt F Fricke 11 May 92
Section IV, Tox. Branch II (H7509C)
Secondary Reviewer: Elizabeth A. Doyle, Ph.D. E.A. Doyle 5/18/92
Section IV, Tox. Branch II (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: In vivo micronucleus test mice (84-2)

P.C. CODE: 128724

MRID NO.: 421698-37

TEST MATERIAL: S-23031

SYNONYMS: Pentyl 2-chloro-4-fluoro-5-[(3,4,5,6-tetrahydro)phthalimido] phenoxyacetate

STUDY NUMBER: 2031

SPONSOR: Sumitomo Chemical Co., Ltd.
Kitahama, 4-Chome 5-33, Chou-Ku
Osaka, Japan

TESTING FACILITY: Sumitomo Chemical Co., Ltd.
Biochemistry and Toxicology Laboratory
1-98, 3-Chome
Kasugade-naka, Konohana-ku
Osaka, Japan

TITLE OF REPORT: Micronucleus Test of S-23031 in ICR Mice

AUTHOR: M. Hara

REPORT ISSUED: 25 May 1990

CONCLUSIONS: The test compound, at the highest dose of 5000 mg/kg, was administered by oral gavage to male and female ICR mice. Compared to the vehicle control, no significant differences in the frequency of micronucleated cells were noted in the bone marrow cells from the animals treated with test compound.

CLASSIFICATION: Acceptable

This study satisfies the guideline (84-2) requirements for a "Structural chromosomal aberration test".

A. **MATERIALS**

1. Test compound: S-23031, technical Description: brown powder Batch #: PYG-88092-M Purity: 94.7% Contaminants: Not given.

2. Control Materials

Negative Control: Corn oil, 20 ml/kg
Positive Control: Cyclophosphamide, 80 mg/kg, dissolved in water and administered in a dose volume of 10 ml/kg.

3. Test animals: Species mouse Strain ICR Age 5 to 6 weeks Weight (g) 36.2-45.6 (male), 26.8-36.6 (female) Source: Japan SLC, Inc., Shizuoka, Japan

B. **TEST PERFORMANCE**

1. Study Design: Two separate studies were performed. A time course study, using 5 animals/sex/time point, evaluated the formation of micronuclei 24, 48 and 72 hours after administration of test compound (5000 mg/kg) by oral gavage. In a second, dose response study, 5 animals/sex/dose were gavaged with test compound at doses of 1250, 2500 and 5000 mg\kg; the formation of micronuclei were evaluated after 24 hours.

2. Cells Examined: Bone marrow cells were examined. For each animal 1000 polychromatic erythrocytes (PCEs) were scored and the frequency of micronucleated cells determined. The ratio of PCEs to whole erythrocytes (PCEs + normochromatic erythrocytes) was also determined.

3. Slide preparation: Bone marrow cells were extracted from the femur with 0.3 ml of fetal bovine serum (FBS) containing 15 mM EDTA. The resulting cell suspension was centrifuged at room temperature for 5 min at 1000 rpm and the resulting cellular pellet resuspended in the remaining residual supernatant. The suspension was smeared onto a clean glass slide and dried overnight. The cells were then fixed with methanol, stained with 5% Giemsa solution, cleared, and dried.

4. Preliminary Toxicity and Clinical Signs: A preliminary range finding study was conducted using five mice per sex per dose (0, 500, 1000, 2500, and 5000 mg/kg). The animals were observed daily for seven days for signs of mortality, moribundity and toxicity. All animals survived through the seven day observation period with no dose-related changes in body weight and body weight gain. Except for soft stools, no other clinical signs were noted. From the results of this preliminary study, 5000 mg/kg was selected as the highest dose used in the micronucleus study.

5. Micronucleus Assay Results: Tables 1 and 2 summarize the results of the time course and dose response studies, respectively. Neither study showed significant differences in any of the treatment-related effects. The percentage of micronucleated cells was comparable to control values; the percentage of PCEs did not vary significantly with treatment. The positive control, however, had significantly higher percentage of micronucleated cells. The percentage of PCEs in the positive control groups was significantly lower than the corn oil control. The percentage of PCEs was significantly lower only in the male animals of the dose response study.

6. Conclusions: The test compound, at the highest dose of 5000 mg/kg, was administered by oral gavage to male and female ICR mice. Compared to the vehicle control, no significant differences in the frequency of micronucleated cells were noted in the bone marrow cells from the animals treated with test compound.

Classification: Acceptable

This study satisfies the guideline (84-2) requirements for a "Structural chromosomal aberration test".

7. Test was performed under GLPs and a quality assurance statement was enclosed in the study.

8. CBI appendix was not attached.

Page_____ is not included in this copy.

Pages 50 through 51 are not included.

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____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

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Reviewed by: Robert F. Fricke, Ph.D. Robert F Fricke 19 May 92
Section IV, Tox. Branch II (H7509C)
Secondary Reviewer: Elizabeth A. Doyle, Ph.D. E A. Doyle 5/20/92
Section IV, Tox. Branch II (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: Mammalian cells in culture cytogenetics assay in Chinese Hamster Ovary (CHO) cells (84-2)

P.C. CODE: 128724 009587

MRID NO.: 421698-38

TEST MATERIAL: S-23031

SYNONYMS: Pentyl 2-chloro-4-fluoro-5-[(3,4,5,6-tetrahydro)phthalimido] phenoxyacetate

STUDY NUMBER: 1875

SPONSOR: Sumitomo Chemical Co., Ltd.
Kitahama, 4-Chome 5-33, Chou-Ku
Osaka, Japan

TESTING FACILITY: Sumitomo Chemical Co., Ltd.
Biochemistry and Toxicology Laboratory
1-98, 3-Chome
Kasugade-naka, Konohana-ku
Osaka, Japan

TITLE OF REPORT: *In vitro* Chromosomal Aberration Test of S-23031 in Chinese Hamster Ovary Cells (CHO-K1)

AUTHOR: S. Kogiso

REPORT ISSUED: 20 November 1989

CONCLUSIONS: The results of this study indicate that in the absence of metabolic activation, the test compound was a weak inducer of chromosomal aberrations; in the presence of metabolic activation, the results were negative.

CLASSIFICATION: Unacceptable

(Study may be upgraded to acceptable if documentation is provided which indicates that the cell cultures were properly maintained and periodically checked for both mycoplasma contamination and karyotype stability.)

This study does not satisfy the guideline (84-2) requirements for a "Structural chromosomal aberration test".

A. MATERIALS

1. Test compound: S-23031, technical Description: brown powder Batch #: PYG-88092 Purity: 94.4% Contaminants: Not given.

2. Control Materials

Negative Control: DMSO, 0.5% final concentration

Positive Control

Nonactivated: Mitomycin C, 0.2 µg/ml, 18 hr harvest
0.1 µg/ml, 24 hr harvest

Activated: Cyclophosphamide, 150 µg/ml, 8 hr harvest
50 µg/ml, 16 hr harvest

Positive Control Solvent: normal saline

3. Activation: S-9 derived from PCB (Kanechlor-400) induced rat liver. The S-9 fraction was stored at -80°C until used.

One ml of S-9 mixture contained 0.35 ml water, 0.1 ml 50 mM $MgCl_2$, 0.1 ml 330 mM KCl, 1.7 mg glucose-6-phosphate, 3.62 mg NADPH, 0.2 ml 20 mM HEPES buffer (pH 7.2), and 0.25 ml S-9 fraction. The S-9 mixture was prepared fresh, before each use.

4. Test Compound Concentrations:

Chromosomal Aberration Assay: 50, 100, 150 and 200 µg/ml for nonactivated conditions and 100, 200, and 400 µg/ml for activated conditions:

Cytotoxicity Assay: 0.78, 1.56, 3.13, 6.25, 12.5, 25, 50, 100, 200, 400 µg/ml for both nonactivated and activated conditions.

5. Indicator Cells: Chinese hamster ovary cells (CHO-K1) (Dainippon Pharmaceutical Co., Ltd., Osaka, Japan) were used as the indicator cells in this study. At the exponential growth phase the doubling time was approximately 14 hr with a modal chromosome number of 20. There is no documentation in the study which indicates that the cells were properly maintained and periodically checked for both mycoplasma contamination and karyotype stability.

6. Study was performed under GLPs and a quality assurance statement was enclosed in the study.

7. CBI appendix was not attached.

B. **TEST PERFORMANCE**

1. Cytotoxicity Assay: Culture medium consisted of Ham's F-12 medium supplemented with 10% fetal bovine serum (FBS), 50 IU/ml penicillin and 50 μg/ml streptomycin. Cells were grown in a monolayer at 37°C in a humidified atmosphere containing 5% CO_2. The cell culture was initiated by seeding approximately 2.5×10^5 cells into 60 mm diameter dish containing 5 ml of culture medium. Twenty-four hours after cultivation the cell count increased to 5×10^5. The nonactivation and activation assays are described as follows:

Nonactivation Assay: A 25 μl aliquot of test compound solution was added to cells in 5 ml of complete medium. Two hr after the start of the incubation with test compound, 5-bromo-2'-deoxyuridine (BrdU) was added to the medium at a final concentration of 2 μg/ml. Cells were cultured for 26 hr in the dark and then harvested.

Activation Assay: After discarding the culture medium, the cells were cultured for two hr in 4.5 ml of FBS-free culture medium containing 0.5 ml of S-9 mixture and 25 μl aliquot of test compound solution. After a two hour incubation, the medium was removed and the cells washed two times with fresh medium to remove the test compound. Five ml of fresh medium containing 2 μg/ml BrdU was added to the cells. The cells were incubated in the dark for 26 hours and then harvested.

2. Chromosomal Aberration Assay: The cell culture was initiated by seeding approximately 2.5×10^5 or 5×10^5 cells into 60 mm diameter dish containing 5 ml of culture medium; after 24 hours the cell counts increased to 6.5×10^5 or 9×10^5, respectively. The dishes containing 6.5×10^5 were used for the nonactivation assay and the 16 hour incubation with activation, while the dishes containing 9×10^5 cells were used for the 8 hour incubation with activation. The cells were treated as follows:

Nonactivation Assay: Twenty five μl of test compound were added to each culture dish and incubated for either 18 or 24 hours. At the end of the incubation period, cells were harvested as described below.

Activation Assay: After discarding the culture medium, the cells were cultured for two hr in 4.5 ml of FBS-free culture medium containing 0.5 ml of S-9 mixture and 25 μl aliquot of test compound solution. Following the exposure period, the cells were washed two times with fresh medium and then replaced with 5 ml of complete medium. The cells were incubated for 8 and 16 hr and harvested as described below.

3. Harvest Procedure: Colcemid, at a final concentration of 0.1 μg/ml, was added for the final 2 hr of the incubation period. At the end of the incubation, the metaphase cells were detached from the culture dish by treating them with a 0.04% trypsin solution in phosphate-buffered saline. After a few minutes, the trypsinized cells were transferred to a test tube and collected by centrifugation (1000 rpm, 5 min). The cells were then swollen in KCl (75 mM) at room temperature and fixed with methanol:acetic acid (3:1) for 20 minutes. The fixed cells were spread onto a clean slide, air-dried, and aged for 24 hr. For the cytotoxicity assay, slides were differentially stained using a modified fluorescence-plus-Giemsa procedure. Slides were stained with Hoechst 33258. For the cytotoxicity assay cells were stained in 3% Giemsa.

For determination of cytotoxicity, 100 cells were examined and the percentage of cells that completed one, between one and two and two cycles in BrdU determined; the mitotic index was also determined. For determination of chromosomal aberrations, 100 cells, containing 20 ± 2 centromeres, were analyzed and the number and type of aberrations noted.

4. Statistical Analysis: The chi-square test was used to evaluate the number of cells with aberrations and the frequency of cells with aberrations.

5. Evaluation Criteria: If the test compound induced a significant, compared to negative control, increase in the frequency of structural aberrations (excluding gaps) and a dose-response relationship or reproducibility existed, then the compound was judged to be clastogenic.

C. **RESULTS**

1. Cytotoxicity Assay: The results of the cytotoxicity assay are summarized in Table 1 of the appendix. At 400 μg/ml, the test compound precipitated out, forming a cloudy suspension. The medium was emulsified at both 100 and 200 μg/ml. Without metabolic activation, the cell cycle was delayed at 100 μg/ml and higher concentrations of test compound. At 200 μg/ml and higher, the mitotic index was lower than the solvent control value. The test compound was cytotoxic at 400 μg/ml. In the metabolic activation study, the highest concentration tested, 400 μg/ml, did not result in any cytotoxicity or cell cycle delay. Based upon these results, the highest concentrations of test compound chosen for the chromosomal aberration assays were 200 and 400 μg/ml without and with metabolic activation, respectively.

2. Chromosomal Aberrations: The results of the chromosomal aberration study without metabolic activation are summarized in the appendix (Tables 2 to 5). For the both 18- and 24-hr incubations, exposure to test compound, at concentrations of 150 and 200 μg/ml, significantly increased both the total

number of aberrations and the percentage of cells with aberrations. The positive control produced significant increases in both the total number of aberrations and the percentage of cells with aberrations.

In the presence of metabolic activation, the only significant finding was an increase in the total number of aberrations and the percentage of cells with aberrations in the 100 µg/ml group (Appendix, Tables 6 to 9). Since higher concentrations of test compound failed to elicit an effect, no dose-response relationship existed. Again, the positive control produced significant increases in both the total number of aberrations and the percentage of cells with aberrations.

D. CONCLUSIONS: The results of this study indicate that in the absence of metabolic activation, the test compound was a weak inducer of chromosomal aberrations; in the presence of metabolic activation, the results were negative.

Classification: Unacceptable

(Study may be upgraded to acceptable if documentation is provided which indicates that the cell cultures were properly maintained and periodically checked for both mycoplasma contamination and karyotype stability.)

This study does not satisfy the guideline (84-2) requirements for a "Structural chromosomal aberration test".

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Pages 157 through 165 are not included.

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Reviewed by: Robert F. Fricke, Ph.D.
Section IV, Tox. Branch II (H7509C)
Secondary Reviewer: Elizabeth A. Doyle, Ph.D.
Section IV, Tox. Branch II (H7509C)

5/18/92

009587

DATA EVALUATION REPORT

STUDY TYPE:	Unscheduled DNA Synthesis in Primary Rat Hepatocytes (84-2)
P.C. CODE:	128724
MRID NO.:	421698-39
TEST MATERIAL:	S-23031
SYNONYMS:	Pentyl 2-chloro-4-fluoro-5-[(3,4,5,6-tetrahydro)phthalimido] phenoxyacetate
STUDY NUMBER:	1929
SPONSOR:	Sumitomo Chemical Co., Ltd. Kitahama, 4-Chome 5-33, Chou-Ku Osaka, Japan
TESTING FACILITY:	Sumitomo Chemical Co., Ltd. Biochemistry and Toxicology Laboratory 1-98, 3-Chome Kasugade-naka, Konohana-ku Osaka, Japan
TITLE OF REPORT:	In vitro Unscheduled DNA Synthesis (UDS) Assay of S-23031 in Rat Hepatocytes
AUTHOR:	S. Kogiso
REPORT ISSUED:	12 December 1989

CONCLUSIONS: The test compound did not fulfil either of the evaluation criteria for a positive result. At concentrations up to 300 µg/ml, the test compound did not elicit unscheduled DNA synthesis in primary cultures of rat hepatocytes.

This study is acceptable and fulfills the guideline (84-2 (3)) requirements for "Other Genotoxic Effects".

CLASSIFICATION: Core - acceptable

A. MATERIALS

1. Test compound: S-23031, technical Description: brown powder Batch #: PYG-88092-M Purity: 94.7% Contaminants: Not given.

2. Control Materials

Negative control: Vehicle (DMSO), 1% final concentration
Positive Control: 2-Acetylaminofluorene (2-AAF), 0.05 µg/ml

3. Test Compound Concentration Used: 1, 3 10, 30, 100 and 300 µg/ml

4. Indicator Cells: Hepatocytes, used as the indicator cells, were obtained from 6 to 7 week old male Sprague-Dawley rats (Charles River Japan, Inc.) weighing 225 to 265 g. Animals were allowed food (CRF-1, Oriental Yeast Co., Ltd., Tokyo, Japan) and water ad libitum.

5. Isolation of Hepatocytes: Primary cultures of hepatocytes were obtained using the modified method of Nakamura (The Methods of Experiments Using Rat Hepatocyte Primary Culture, in Japan Scientific Societies Press, pp 5-53, 1987).

a. Liver Perfusion: After cannulation of the portal vein, the liver was perfused in situ with perfusion buffer (Table 1) for five minutes. After five minutes, the liver was perfused with collagenase solution (Table 1) for 10 minutes.

b. Cell Isolation and Culturing: The perfused liver was removed and placed in an ice-cold solution of William's E medium (supplemented with 50 IU/ml of penicillin and 50 µg/ml of streptomycin) containing 10% fetal bovine serum (WmE/FBS). The tissue was then minced into small pieces and filtered through 4 layers of sterile gauze. The filtrate was centrifuged (1 min, 50 x g) at 4°C. After discarding the supernatant fraction, the packed cells were washed and recentrifuged two times using ice-cold WmE/FBS medium. The final pellet was resuspended in 30 ml of WmE/FBS medium.

Following determination of cell viability (trypan blue exclusion method), the concentration of live cells in the suspension was adjusted to 2.5×10^5 cells/ml. Two ml of cell suspension was then pipetted into the wells of 6-well culture dishes containing a 25 mm round cover slip. Cells were allowed to attach to the cover slip during a 2 hour incubation at 37°C under an atmosphere of 5% CO_2.

B. **TEST PERFORMANCE**

1. Dose Selection: Dose selection was based upon an initial cytotoxicity assay. Hepatocytes were incubated (18 hours, 37°C, 5% CO_2) with test compound at concentrations of 1 to 300 µg/ml of medium. Following the incubation the cells were washed and viability assessed using the trypan blue exclusion method. The selection of the highest concentration (300 µg/ml) of test compound was based upon the limit of solubility.

2. UDS Assay

a. Treatment: Following the initial two hour incubation, the culture medium was removed and replaced with 2 ml of WmE medium containing 20 µl of solution of appropriate concentration of test compound and H^3 thymidine (370 Kbq/ml, specific activity = 925 GBq/mmole). The solvent and positive control (2-AAF, 0.05 µg/ml) were run concurrently. Hepatocytes were incubated for 18 hours at 37°C under an atmosphere of 5% CO_2. Each concentration was assayed in duplicate; the results confirmed by a repeat assay.

b. Slide Preparation: Following the incubation, the cells were washed, treated for 10 minutes with 2 ml of 1% sodium citrate, and fixed three times with 2 ml of ice-cold ethanol:acetic acid (3:1) for 30 minutes each time. The cover slips were immersed in 99.5% ethanol, air dried, and mounted on glass slides.

b. Preparation of Autoradiographs/Grain Development: Slides were dipped in Kodak NTB-2 photographic emulsion at 40°C, dried, and placed in a cool (4°C) light-tight box for 1 week. The exposed slides were developed for five minutes, immersed in 2% acetic acid, fixed, and rinsed with water. All slides were stained using Meyer's hematoxilin and eosin.

c. Grain Counting: The area of grains was automatically counted and mathematically transformed to grain counts. If possible, at least 50 cells per slide were counted for each dose.

3. Evaluation Criteria: To be a positive result, the percentage of cells in repair must be greater than or equal to 20% or the net grain counts must be greater than zero and be significantly different from the solvent control. The increases in number of cells in repair and the net grain counts must be dose-dependent.

4. The test was performed under GLP's; a quality assurance statement is present in the study.

5. CBI appendix is not attached.

C. **REPORTED RESULTS**

Viability (relative to the solvent control) was 66.2% for the 300 µg/ml concentration; incubation with 100 or 30 µg/ml resulted in relative viabilities of 88.5 and 99.3%, respectively.

Table 2 summarized the performance of test compound in the UDS assay. At doses of 1, 3, 10, 30, 100, and 300 µg/ml, the test compound did not significantly increase either the net grain counts or the number of cells in repair. The positive control elicited positive response with significant increases in both the number of net grain counts and the number of cells in repair. The results were confirmed in a repeat study.

D. **CONCLUSIONS**

The test compound did not fulfil either of the evaluation criteria for a positive result. At concentrations up to 300 µg/ml, the test compound did not elicit unscheduled DNA synthesis in primary cultures of rat hepatocytes.

This study is acceptable and fulfills the guideline (84-2 (3)) requirements for "Other Genotoxic Effects".

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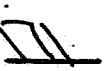
Pages  through  are not included.

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Reviewed by: Robert F. Fricke, Ph.D. Robert F. Fricke 30 June 92
Section IV, Tox. Branch II (H7509C)
Secondary Reviewer: Elizabeth A. Doyle, Ph.D. E. A. Doyle 6/30/92
Section IV, Tox. Branch II (H7509C)

DATA EVALUATION REPORT 009587

STUDY TYPE: Metabolism - Pharmacokinetics in rats (85-1)

P.C. CODE: 128724

MRID NO.: 421698-40

TEST MATERIAL: S-23031

SYNONYMS: Pentyl 2-chloro-4-fluoro-5-[(3,4,5,6-tetrahydro)phthalimido]phenoxy acetate

STUDY NUMBER: 1918

SPONSOR: Valent U.S.A. Corporation
1333 N. California Blvd
Walnut Creek, CA 94596

TESTING FACILITY: Sumitomo Chemical Co., Ltd
Biochemistry and Toxicology Laboratory
4-2-1, Takatsuasa, Takarazuka
Hyogo, Japan

TITLE OF REPORT: Metabolism of S-23031 in Rats Revised

AUTHOR: N. Isobe

REPORT ISSUED: 2 August 1990, revised 6 December 1991

CONCLUSIONS: The absorption, distribution, metabolism and excretion of [phenyl-(UL)^{14}C]-labeled test compound was studied on three groups of male and female rats. Two study groups received a single dose by oral gavage of labeled test compound at either 1 mg/kg or 500 mg/kg; the third group was treated daily for 14 days with unlabeled test compound at 1 mg/kg/day by oral gavage, followed on the 15th day with a dose of 1 mg/kg of labeled compound. For seven days after the administration of labeled test compound, urine and feces were collected. On Day 7 the animals were necropsied and selected tissues analyzed for radioactivity.

The test compound is rapidly absorbed and eliminated; after 48 hours, 92.7 to 97.8% of the administered radioactivity was recovered in the urine and feces. The distribution of labeled residues between the urine and feces was comparable in the low and repeat dose groups. However, for animals in the high dose group, fecal elimination of the test compound predominated.

The metabolic profiles for were similar for all three study groups. The primary metabolic transformation was deesterification. The deesterified residues were either cleaved of the imide moiety or underwent a series of hydroxylation and/or sulfonation reactions. Compared to the low dose group, the repeat dose group showed slightly higher sulfonation, although the difference was not significant.

The tissue accumulation of ^{14}C-labeled residues was very low. Detectable amounts were found in the only kidneys and livers. Accumulation of residues in the kidneys of the females was significantly higher than the males.

Classification: core - Supplementary. This study may be upgraded if the following additional information is provided and is judged to be acceptable:

1. Although the major metabolites which contain the phenyl ring have been characterized, metabolites containing the phthalimide moiety have not. Since cyclohexenedicarboximide and its metabolites account for approximately 30% of the residues present, failure to identify them is a serious deficiency of this study.

2. The specification sheet for the analysis of labeled test compound was not given in the study. While the text referenced lot no. C-88-024, the specification sheet in appendix A lists the lot no. as C-88-025.

3. No information is given in the methods section describing how the two 4-OH-IMCA-SA isomers and 5-OH-IMCA-SA were isolated and identified. In the Results and Discussion section the study states on page 18 that "Presence of these three metabolites was confirmed by high performance liquid chromatography" and referenced an unpublished observation.

4. The data on biliary excretion of the labeled compound is needed; it not sufficient to cite "unpublished observations". If the observations are important enough to be mentioned in the study, the data should have been included as an appendix of the report.

This study does not satisfy guideline requirements (85-1) for a metabolism study in rats.

MATERIALS AND METHODS

A. Materials

1. Test compound

[phenyl-(UL)^{14}C] S-23031
Lot No.: see note
Radiochemical purity: >99%
Specific Activity: 195 mCi/mmole, 0.459 mCi/mg

Note: The lot number of the [phenyl-(UL) ^{14}C] S-23031 given page 4 of the study (C88-024) does not correspond with the lot number given on the specification sheet in Appendix A of the study (C88-025).

Nonradioactive S-23031
Lot No.: LN-80206
Purity: 99.2%

2. Vehicle: Corn oil

3. Test animals: Sprague-Dawley rats were obtained from Charles River Japan, Co. The body weights and ages of the animals are given in Table 1.

Table 1: Body weights and ages of test animals

Group	Body Weight (g) Males	Females	Age (weeks)
Low Dose	251-260	173-179	7
High Dose	256-263	163-178	7
Repeat Dose	123-128	113-119	5

B. Study Design:

1. Group Assignments and Dosing: Animals, 5/sex/group, were assigned to low, high and repeat dose study groups. The low (1.0 mg/kg, 0.5 μCi/mg) and high dose (500 mg/kg, 250 μCi/mg) groups each received a single dose of test compound by oral gavage. Animals in the repeat dose group were dosed orally with unlabeled test compound (1 mg/kg) for 14 consecutive days. On the 15th day, animals received a single oral dose of labeled test compound (1 mg/kg, 0.5 μCi/mg). Following the administration of labeled compound, the animals were placed individually in metabolic cages.

2. Analysis of Fecal and Urinary Samples: Fecal and urinary samples were collected 6 hr (urine only), 1, 2, 3, 5, and 7 days after administration of labeled compound.

Cages were rinsed to remove any residual radioactivity, which was included in the urine radioactivity. Duplicate aliquots of urine were analyzed for radioactivity. Fecal samples, collected after Day 2, were homogenized in water and the radioactivity determined on a combusted sample. Fecal samples, collected during the first two days of the study, were extracted as described below in section 4 (Identification of Major Metabolites).

3. Tissue Distribution of Radioactivity: At terminal sacrifice the adrenals, bone, bone marrow, brain, fat, heart, kidney, liver, lung, muscle, spleen, pancreas, thyroid and testes (male) or ovary and uterus (female) were collected. Samples of each tissue, minced carcass and blood were combusted for determination of radioactivity.

4. Identification of Major Metabolites: The major metabolites and their abbreviations are given in Table 2. Two standards (AFCA and IMCA) were synthesized and the structures confirmed by NMR and mass spectroscopy. Five other standards were prepared from extracts of the fecal and urinary samples. Referencing an unpublished observation, cited in the study as reference 1, the study indicates that the unidentified urinary and fecal residues were separated on thin layer chromatography (TLC), purified and the chemical structures determined by NMR, infrared and mass spectroscopic analyses. For completeness of the study, the analytical procedures used for separation and identification of the residues should have been included in the study. It is not readily apparent how the two 4-OH-IMCA-SA isomers and 5-OH-IMCA-SA were purified.

To identify the ^{14}C-labeled residues, the fecal and urinary samples for days 0 to 2 were combined. The urinary samples were concentrated in a rotary evaporator, while the individual fecal samples were homogenized in 50 to 100 ml of acetonitrile. The homogenate was centrifuged and the total volume of the supernatant determined. The remaining pellet was extracted two more times with acetonitrile and three times with methanol:water (9:1, v/v). Since more than 10% of the radioactivity remained in the pellet of the low dose and repeated dose groups, these samples were extracted twice more with water. For each animal, the different extracts were concentrated on a rotary evaporator and pooled. Aliquots of the concentrated urine and fecal extracts were spotted onto two silica gel plates, one plate was developed with toluene:ethyl formate:formic acid (5:7:1) and the other with 1-butanol:acetic acid:water (6:1:1). The radioactivity on the plates was detected by autoradiography using SB-5 film (Kodak). To aid in the identification of the residues, authentic standards were co-chromatographed with the experimental samples.

Table 2: Metabolites of the test compound and their abbreviations (Taken from Table 1 of the study)

S-23031	Pentyl 2-chloro-4-fluoro-5-[(3,4,5,6-tetrahydro)-phthalimido]phenoxyacetate
AFCA	(2-chloro-4-fluoro-5-amino)phenoxyacetic acid
IMCA	[2-chloro-4-fluoro-5-(3,4,5,6-tetrahydro) phthalimido]phenoxyacetic acid
4-OH-IMCA	[2-chloro-4-fluoro-5-(4-hydroxy-1,2-cyclohexene-dicarboximido)]phenoxyacetic acid
IMCA-SA	[2-chloro-4-fluoro-5-(1-sulfo-1,2-cyclohexane dicarboximido)]phthalimido)phenoxyacetic acid
4-OH-IMCA-SA[a]	[2-chloro-4-fluoro-5-(1-sulfo-4-hydroxy-1,2-cyclohexanedicarboximido)]phenoxyacetic acid
5-OH-IMCA-SA	[2-chloro-4-fluoro-5-(1-sulfo-5-hydroxy-1,2-cyclohexanedicarboximido)]phenoxyacetic acid

a. Study indicated that this metabolite was present as two stereoisomers.

5. Statistics: Significant differences between sexes or dose groups was determined using Student's t-test. Outliers were determined using Smirnov-Grubbs test at 1% probability level.

6. Quality assurance was documented by signed and dated GLP and quality assurance statements.

Results

A. Clinical Observations: No treatment-related clinical signs of toxicity were seen in animals in any of the treatment groups.

B. Distribution of Radioactivity in Excreta: A preliminary experiment evaluated the distribution of labeled test compound in the feces, urine and expired air. After administration of test compound, labeled with ^{14}C on either the phenyl or tetrahydro-phthaloyl moieties, no radioactivity was detected in the expired air. Essentially all (97.1 to 100.1%) of the administered ^{14}C dose could be accounted for in the urine and feces. Therefore, for the main study only the urine and feces were collected for routine analysis of ^{14}C-labeled residues.

The cumulative amounts of radioactivity in the feces and urine are summarized in Table 3. For all three study groups 92.7 to 98.7% of the administered radioactivity was excreted within the first two days. In the low and repeat dose groups, the

Table 3: Cumulative amounts of ^{14}C-labeled residues in feces and urine (Data taken from Tables 3-1, 3-2 and 3-3 of the study)

		Cumulative % of Dosed ^{14}C in Excreta after					
		6 hr	1 day	2 days	3 days	5 days	7 days
Low Dose Group							
Males	Feces	---	49.8	55.6	55.7	55.8	55.9
	Urine	17.1	38.3	39.2	39.2	39.3	39.3
	Total	17.1	88.0	94.7	95.0	95.1	95.2
Females	Feces	---	37.5	43.0	43.1	43.2	44.1
	Urine	28.5**	50.8**	52.1**	52.3**	52.4**	52.6**
	Total	28.5	88.3	95.1	95.4	95.6	96.7
High Dose Group							
Males	Feces	---	70.8	77.5	77.5	77.9	78.0
	Urine	6.6	20.2	21.3	21.5	21.7	21.7
	Total	6.6	91.0	98.7	99.3	99.6	99.7
Females	Feces	---	48.8	57.1	57.4	57.4	57.4
	Urine	18.6**	34.1**	35.6**	35.7**	35.9**	36.3**
	Total	18.6	82.9	92.7	93.1	93.3	93.7
Repeat Dose Group							
Males	Feces	---	50.8	57.3	57.5	57.5	57.5
	Urine	18.6	38.7	39.7	39.8	39.9	40.0
	Total	18.6	89.4	97.0	97.3	97.4	97.5
Females	Feces	---	43.7	48.3	48.9	48.9	48.9
	Urine	24.1*	48.3*	49.0*	49.3*	49.4*	49.5*
	Total	24.1	92.0	97.8	98.2	98.3	98.4

* $p \leq 0.05$, ** $p \leq 0.01$

distribution of the excreted ^{14}C-labeled residues between the feces and urine showed a slight, sex-related difference. Fecal elimination was higher in males, while in females, urinary elimination predominated. Within each sex the distribution of labeled residues between the feces and urine was comparable. In the high dose group, the feces was the major route of elimination in both sexes, but, as with the other two dose groups, urinary elimination predominated in the females (40% vs. 22%).

C. Tissue Distribution of Radioactivity: At terminal sacrifice, the percentage of radioactivity still present in the body was low (0.03 to 1.2%) and most organs had no detectable radioactivity (Table 4). However, kidneys from animals in the low and repeat dose groups had 0.8 and 2.4 ppb of ^{14}C-labeled residues for males and females, respectively. In the high dose group, residues

present in the kidney equaled 210 and 340 ppb for males and females, respectively. In all cases, labeled residues in the kidney were significantly higher in the females. For both the low and repeat dose groups, barely detectable levels (0.04 and 0.03 ppb for males and females, respectively) of residues were found in the liver; the high dose group showed detectable levels (190 ppb) only in the male livers.

D. Identification of Major Metabolites: Characterization of the ^{14}C-labeled residues was performed on pooled, 0 to 2 day urine samples and fecal extracts and is summarized in Table 5. For the low and repeat dose groups the parent compound is largely metabolized with very little appearing in the feces (0.1 to 0.7%, respectively) and undetectable amounts in the urine. In the high dose group, unmetabolized parent compound was present in large amounts in the feces (27.7% for females and 35.2% for males); the urine output of parent compound was negligible.

The parent compound is metabolized by two pathways (Figure 1). In the first pathway, the parent compound is deesterified to phenoxyacetic acid derivative, which then undergoes a series of hydroxylation and/or sulfonation reactions to form 4-OH-IMCA, IMCA-SA, two 4-OH-IMCA-SA isomers and 5-OH-IMCA-SA. IMCA-SA, two 4-OH-IMCA-SA isomers and 5-OH-IMCA-SA were primarily eliminated in the feces and to a lesser extent, the urine. The elimination of 4-OH-IMCA was approximately equal in both the feces and urine. Although two metabolites (IMCA-SA and 4-OH-IMCA) could clearly be separated on TLC, the other three (two 4-OH-IMCA-SA isomers and 5-OH-IMCA-SA) co-migrated with identical R_f values in four different solvent systems. The study indicated that separation of co-migrating residues was carried out using HPLC, however, data were not included in the study to substantiate the claims.

In the second pathway, the parent compound is cleaved and deesterified to form AFCA and 1,2-cyclohexenedicarboximide. The AFCA is primarily eliminated in the urine. The metabolic fate of the cyclohexenedicarboximide residue could not be determined since it was not radiolabeled.

Discussion and Comments

The metabolism of [phenyl- (UL) ^{14}C]-labeled test compound was studied on three groups of male and female rats. Two study groups received a single dose by oral gavage of labeled test compound at either 1 mg/kg or 500 mg/kg. The third group was treated daily with unlabeled test compound at 1 mg/kg/day by oral gavage, followed on the 15th day with a dose of 1 mg/kg of labeled compound. Following the administration of labeled test compound, urinary and fecal samples were collected and analyzed for radioactivity. The urinary and fecal samples through Day 2 of the study were pooled and metabolic profiles determined for all three study groups. At the end of seven days, the animals were sacrificed and the amount of radioactivity in the tissues, blood and carcass was determined.

Table 4: Tissue Distribution of ^{14}C-labeled residues. (Compiled from Tables 4-1, 4-2 and 4-3 of the study)

	ng of S-23031 Equivalents/g Tissue					
	Low Dose		High Dose		Repeat Dose	
Tissue	Male	Female	Male	Female	Male	Female
Adrenals	<1.7	<1.2	<640	<490	<1.7	<1.4
Blood	0.4	0.5	<140	<130	0.5	<0.3
Erythrocytes	0.5	0.4	160	150	<0.3	<0.3
Bone	<0.3	<0.3	<130	<140	<0.3	0.5
Bone marrow	<1.2	<2.1	<800	<950	<2.1	<3.1
Brain	<0.3	<0.3	<140	<140	<0.3	<0.3
Fat	<0.8	<0.6	<290	<310	<0.9	0.8
Heart	<0.3	<0.4	<140	<140	0.4	<0.3
Kidney	0.8	2.4**	210	340**	0.8	2.4**
Liver	0.4	0.3	190	<140	0.4	0.3
Lung	<0.3	0.5	<130	<140	<0.3	<0.3
Muscle	<0.3	<0.3	<130	<130	<0.3	<0.3
Ovary	---	<0.7	---	<390	---	<0.9
Pancreas	<0.3	<0.3	<130	<130	<0.3	<0.3
Plasma	0.6	<0.3	240	<140	0.7	<0.3
Spleen	<0.3	<0.3	<130	<140	<0.3	<0.4
Testis	<0.3	---	<140	---	<0.3	---
Thyroid	<4.6	<3.4	<1960	<2240	<4.0	<5.0
Uterus	---	0.9	---	<170	---	<0.4
Carcass	0.7	0.4	770	260	0.3	0.4

** $p \leq 0.01$

Essentially all of the administered radioactivity could be accounted for in the excreta, tissues and carcass. The low and repeat dose animals had comparable distribution of radioactivity in the urine and feces, suggesting that there is no significant bioaccumulation and that the test compound does not induce microsomal enzymes. Animals in the high dose group showed a large amount of unmetabolized test compound in the feces. Since the study stated that biliary excretion does not take place, the test compound must not be completely absorbed from the gastro-intestinal tract.

Of the two metabolic pathways, this study characterized the metabolites which contained the phenoxyacetate moiety. From the scheme presented, the parent compound is deesterified followed by a series of hydroxylation and/or sulfonation reactions. The second, and probably more important pathway, involved the cleavage of the parent compound to form AFCA and cyclohexenedicarboximide. Failing to evaluate the metabolism of cyclohexenedicarboximide is a serious shortcoming of this study

Table 5: Metabolite profiles (% of dosed ^{14}C) in feces and urine (Data taken from Tables 5-1, 5-2 and 5-3 of the study)

	Low Dose Group				High Dose Group				Repeat Dose Group			
	Males		Females		Males		Females		Males		Females	
Metabolite	Feces	Urine	Feces	Urine	Feces	Urine	Feces	Urine	Feces	Urine	Feces	Urine
S-23031	0.4	<1.0	0.7	<1.0	35.2	<1.0	27.7	<1.0	0.1	<1.0	0.1	<1.0
IMCA	9.5	0.9	7.1	1.8	14.0	0.5	6.7	6.4	4.8	0.3	5.0	1.6
AFCA	4.8	22.5	3.9	29.8	6.3	14.3	5.1	20.8	4.1	25.4	3.7	29.2
4-OH-IMCA	2.0	1.8	1.2	0.9	1.5	0.4	1.0	1.3	2.0	0.9	1.4	2.4
IMCA-SA	9.4	1.2	7.4	0.9	3.1	0.2	3.0	0.3	15.3	0.7	14.2	0.3
4-&5-OH-IMCA-SA[a]	12.9	0.8	9.0	0.2	5.9	0.3	0.1	0.2	16.3	0.4	10.0	0.2
UM-4[b]	1.2	1.0	<1.0	1.9	---	---	---	---	1.2	<1.0	1.1	<1.0
UM-5	1.2	<1.0	<1.0	3.7	1.4	2.6	1.0	<1.0	<1.0	5.0	<1.0	5.6
UM-6	<1.0	<1.0	<1.0	1.7	---	---	---	---	<1.0	<1.0	<1.0	1.4
UM-7	1.8	<1.0	1.3	<1.0	---	---	---	---	<1.0	<1.0	<1.0	<1.0
Others[c]	6.2	5.7	4.7	11.1	8.9	3.0	8.1	6.6	8.1	6.9	6.2	8.4

a. 4-OH-IMCA-SA was presumed to be stereoisomers, accurate configuration was not determined. 5-OH-IMCA-SA co-migrated with 4-OH-IMCA-SA in all of the solvent systems used for TLC. The value in the table represents the totals of both isomers of the 4-OH metabolite and the 5-OH metabolite.

b. UM = unknown metabolite

c. Others represents the sum of percentages of unknown metabolites, each of which was present at less than 1.0%.

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since it could be classified as a major metabolite (equimolar with AFCA, which is the most abundant metabolite).

The tissue accumulation of ^{14}C-labeled residues in was very low. Detectable amounts were found only in the kidneys and livers. Accumulation of residues in the kidneys of the females was significantly higher than the males.Figure 1: Proposed metabolic pathway for test compound (Figure copied from page 36 of the study)

Reviewer's Comments: Specific deficiencies are as follows:

1. Although the major metabolites which contain the phenyl ring have been characterized, metabolites containing the phthalimide moiety have not. Since cyclohexenedicarboximide and its metabolites account for approximately 30% of the residues present, failure to identify them is a serious deficiency of this study.

2. The specification sheet for the analysis of labeled test compound was not given in the study. While the text referenced lot no. C-88-024, the specification sheet in appendix A lists the lot no. as C-88-025.

3. No information is given in the methods section describing how the two 4-OH-IMCA-SA isomers and 5-OH-IMCA-SA were isolated and identified. In the Results and Discussion section the study states on page 18 that "Presence of these three metabolites was confirmed by high performance liquid chromatography" and referenced an unpublished observation.

4. The data on biliary excretion of the labeled compound is needed; it not sufficient to cite "unpublished observations". If the observations are important enough to be mentioned in the study, the data should have been included as an appendix of the report.

Classification: core - Supplementary

This study does not satisfy guideline requirements (85-1) for a metabolism study in rats.